DENITRIFICATION IN SMALL RESERVOIRS: UNDERSTANDING NITROGEN REMOVAL ACROSS AN AGRICULTURAL WATERSHED

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ABSTRACT

As the world's increasing human population demands greater agricultural intensity, concerns regarding environmental nutrient loading are also rising. Landowners in the Tobacco Creek Model Watershed (TCMW) in southern Manitoba, Canada, have implemented beneficial management practices to reduce nutrient loading to surface waters. Among these practices is a network of small reservoirs that have been shown to reduce nutrient concentrations downstream. It is unclear what is contributing to this ecosystem service, but bacterial denitrification is thought to be important. To understand the role of these reservoirs in excess nitrate removal and assess how this ecosystem service can be optimized, denitrification activity in reservoirs and stream pools of the watershed was measured via the chloramphenicol-amended acetylene block technique. Results indicated that denitrification activity was positively correlated with measured nitrate concentrations and sediment organic carbon (SOC), and negatively correlated with sediment particle size and pH. Regression trees typically identified nitrate as the primary node in partitioning denitrification activity. Importantly, reservoirs exhibited higher denitrification activity and were at times less likely to be nitrate-saturated than stream pools. This finding was attributed mainly to higher levels of SOC, and lower concentrations of dissolved oxygen found in reservoirs. Nitrate was added to a set of samples to test for nitrate saturation, which is an indicator of poor ecological status, as nitrate concentrations exceed the denitrification capacity of microbes. Almost half of measurements (49%) demonstrated nitrate saturation, indicative of the need for additional remediation activity in the watershed. Classification trees suggested a threshold for nitrate saturation across sites at 0.68 mg L^{-1} NO_3 + NO_2. Findings from this research reveal that reservoirs not only improve nitrate removal capacity, but could inform the proposed construction of additional reservoirs in the TCMW. Understanding nutrient retention in this system could also have implications for downstream ecosystems such as Lake Winnipeg; an intensely eutrophic lake that has become a high priority area for remediation.

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Saskatchewan Water Chemistry and Ecology Laboratory

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LIST OF ABBREVIATIONS

BMPs - Beneficial Management Practices

DOC – Dissolved Organic Carbon

DSWMA - Deerwood Soil and Water Management Association

N_2 – dinitrogen or nitrogen gas

N_2O – nitrous oxide

NH_3 - ammonia

NO_3 + NO_2 – nitrate and nitrite

SOC – Sediment Organic Carbon

SRP – Soluble Reactive Phosphorus

STCW - South Tobacco Creek Watershed

TCMW - Tobacco Creek Model Watershed

TP – Total Phosphorus

USDA – United States Department of Agriculture

WEBs - Watershed Evaluation of Beneficial Management Practices

1.0 GENERAL INTRODUCTION

Conventional agricultural practices apply great amounts of nitrogen and phosphorus to the land in order to encourage high crop yields. Large portions of these nutrients are lost to the environment through leaching to groundwater, runoff to surface waters, and in the case of nitrogen, gaseous emissions (Carpenter et al., 1998; Chambers et al., 2001; Galloway et al., 2003). Nutrient leaching and runoff can degrade the environment, causing eutrophication, and biodiversity loss (Vitousek et al. 1997; Howarth et al., 2000; Chambers et al., 2001).

Beneficial management practices (BMPs) strive to reduce these harmful effects by managing the form, quantity, and timing of nutrient inputs into agricultural systems, and by intercepting the transport of nutrients to watercourses (Pionke et al., 2000; Cherry et al., 2008). Beneficial management practices show promise in addressing issues of nutrient loading. They can, however, be difficult to implement due to great variance in site characteristics (topography, soil, and hydrological pathways [Higgs et al., 2000]) which cause varying efficacy. Numerous BMPs are available for different purposes, and even if the scope is narrowed to address nitrogen loading via surface runoff, there are still many options and factors to consider.

A few common BMPs that address nitrogen in runoff include reduced tillage practices, riparian buffer zones, and constructed wetlands or reservoirs. Each of these practices has been shown to significantly reduce nitrogen exports. For example, conservation tillage has reduced nitrogen export as much as 75% in the southern United States (Sharpley and Smith, 1994), while grassed buffer strips have led to 47 – 100 % reduction in nitrate export in France (Patty et al., 1997). Constructed wetlands have been credited with removal of 98% of ammonium-nitrogen in sites in Ireland (Harrington and McInnes, 2009). If we consider a cold-climate, such as the Canadian prairies, where snowmelt is the main driver of nutrient export, we see these BMPs behaving differently. Tiessen et al. (2010) found that conservation tillage, while reducing nitrogen export to surface waters, increased phosphorus exports, due to interactions with the soil surface, snow cover, and subsequent snowmelt. These same snowmelt and frozen ground conditions could also cause poor functionality in riparian buffer zones, exacerbated by a shorter growing season for riparian vegetation (Oberts, 2003).

The use of wetlands and reservoirs show greater promise as BMPs in prairie systems. Tiessen et al. (2011) reported reductions in total nitrogen and total phosphorus loads up to 20% and 12% respectively, at reservoir structures in the South Tobacco Creek Watershed (STCW) site in Manitoba. These values are lower than those cited in warmer climates, suggesting that reservoirs in prairie systems may not function as effectively. Furthermore, there were a few instances in Tiessen et al.'s (2011) study when the reservoirs acted as sources of nitrogen to downstream water sources, spurred by rainfall and snowmelt events. Suspended nitrogen was not retained as successfully as dissolved nitrogen, suggesting perhaps that nitrogen retention is mainly a factor of plant uptake or bacterial denitrification, rather than sedimentation (Tiessen et al., 2011). In Germany, Paul (2003) found high nitrate removal in a reservoir with low phytoplankton growth, and concluded that nitrogen removal was due to denitrification. Paul (2003) comments on the unexpectedly high denitrification levels, but the actual process of denitrification was not studied in the reservoir. In both Tiessen et al. (2011) and Paul (2003), denitrification activity was theorized as a large player in nitrogen removal, but was not explicitly studied.

Denitrification is the microbial reduction of nitrate to nitrogen gas under low oxygen conditions; an environmental service that provides the only means of permanent nitrogen removal from an ecosystem (Knowles, 1982). The pathway of this reduction produces NO and N_2O as intermediates (Figure 1.1). Genes for denitrification are found across a wide range of microbial genera, with a large suite of heterotrophic bacteria completing the process in aquatic sediments (Zumft, 1997). With such high representation amongst bacteria, it is not surprising that denitrification can be very important in some soils and sediments, with high rates often found in agricultural areas (Inwood et al., 2005; Arango and Tank, 2008; Herrman et al., 2008). Denitrification, however, can also become saturated, limiting the capacity for further nitrogen removal (Bernot and Dodds, 2005; Herrman et al., 2008). Saturation occurs when nitrate is available in excess of one or more of the other factors necessary for the process.

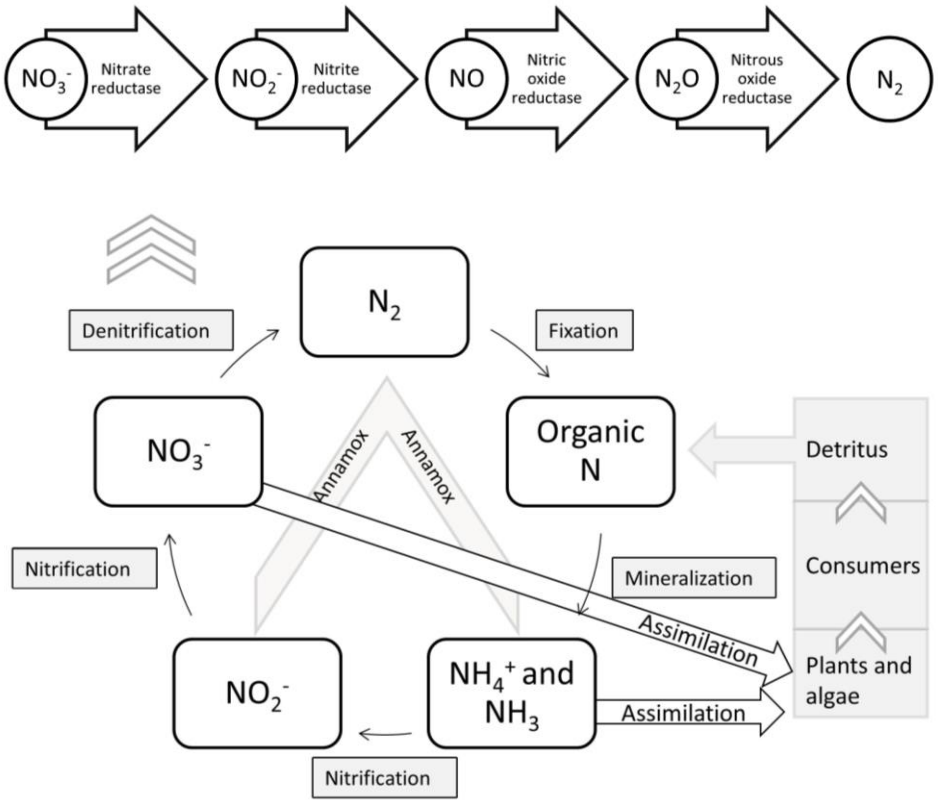

Figure 1.1 Pathway of sequential nitrate reduction via microbial denitrification within the nitrogen cycle

Several factors affect denitrification. Nitrogen oxides, such as nitrate and nitrite, provide an electron acceptor for the process, and are reduced by a series of enzymatic reductases (Figure 1.1). Oxygen represses these enzymes necessary for the sequential reduction of nitrate, such that denitrification occurs only in low oxygen or anoxic conditions (Knowles, 1982). Organic carbon provides available electrons to denitrifying microbes, fueling the denitrification process, and is necessary for its completion. Temperature and pH can govern enzymatic activity of denitrifiers, with nitrate reductase thought to be inhibited below 5°C (Bailey & Beauchamp, 1973) and nitrate consumption reduced at low pH (Baeseman et al., 2006). Many factors interact to affect denitrification as well. For example, primary production increases sediment organic carbon (SOC) via decomposition of biomass, which increases oxygen demand as aerobic microbes grow utilize this carbon source, thereby creating low-oxygen conditions suitable for denitrifying bacteria. In addition, particle size and associated porosity of sediments affect penetration of oxygen, and finer particles with can better retain particle-bound nutrients, such as the organic carbon needed for the process (Brady & Weil, 2008).

There is great variability in the effects of these factors on denitrification rates (Pattinson et al., 1998; Inwood et al., 2005; Arango and Tank, 2008; Herrman et al., 2008). For example, Inwood et al. (2005) found the availability of nitrate to be the most important factor in denitrification rates, except in agricultural streams, where they were unable to definitively say what the limiting factor was. They found higher denitrification rates in warmer summer months, and hypothesized that this observation might be due to temperature in conjunction with other parameters. Denitrification assays in this experiment, however, were conducted in a laboratory under room temperature, and therefore the authors could not determine the effect of temperature on denitrification rates. Under varying experimental conditions, temperature has been found to be important, with increased temperatures generally favoring denitrification (Pattinson et al., 1998; Herrman et al., 2008).

There is also variance in the relationship between organic carbon and denitrification rates. This relationship is complicated due to the ability of denitrifying bacteria to utilize both particulate and dissolved carbon (Inwood et al., 2005). Arango and Tank (2008) identified SOC as the most important predictor of denitrification rates. In contrast, Herrman et al. (2008) found that neither the quality nor quantity of organic carbon affected denitrification rates. Their findings may have reflected the high SOC concentrations in the samples, which can make organic carbon non-limiting and render dissolved organic carbon (DOC) additions insignificant.

These multiple factors affecting denitrification, combined with variation in environmental conditions create challenges in predicting denitrification rates. Further complicating assessment is the difficulty of measuring denitrification. There are several methods available to quantify the process, each with limitations. The chloramphenicol-amended acetylene-block technique, explained elsewhere in this thesis, is most appropriate for this study due to the ability to inexpensively and simply estimate denitrification over a wide area. The acetylene-block method, although historically the most common, has been criticized for inhibition of the nitrate-supplying nitrification process via acetylene (Hynes and Knowles, 1978; Walter et al., 1979), microbial decomposition of acetylene, contamination of acetylene by other gases that affect denitrification, overestimations due to anoxic conditions in the laboratory, and potentially incomplete inhibition of nitrous oxide (N_2O) reductases (Seitzinger et al., 1993; Groffman et al., 2006).

While the acetylene-block method has established weaknesses, many of the issues arise in systems with low nitrate concentrations, and are unlikely to be significant issues in agriculturally affected systems. Addition of chloramphenicol inhibits production of new enzymes that may otherwise be produced in ideal laboratory conditions, allowing estimates of denitrification rates to be as close to *in situ* processes as possible. With this improvement, the method provides good estimates of aquatic denitrification rates, especially in systems with high nitrate, and remains the only feasible method for broad-scale assessment across multiple sites (Groffman et al., 2006).

Other methods often used to estimate denitrification include the use of ^{15}N tracers, direct N_2 measurements, mass balance approaches, and stable isotope abundance measurements. The ^{15}N tracer method is a laborious and expensive procedure, and assumes that there is no increase in denitrification due to the added labeled substrates (Groffman et al., 2006). Direct N_2 quantification methods, such as membrane inlet mass spectrometry, require long pre-incubations and flushing with a gas-tight apparatus, making design, construction and operation of this method complex (Seitzinger et al., 1993; Groffman et al., 2006). Membrane inlet mass spectrometry is therefore unsuitable for studies requiring a large number of samples across a relatively large area. Membrane inlet mass spectrometry can also lead to underestimation of rates of N_2 production due to reactions between nitrogen and oxygen within the mass spectrometer (Eyre et al., 2002). This method may also underestimate denitrification if reduction of nitrogen oxides stops at an intermediate step rather than continuing to completion (Bernot et al., 2003).

The mass balance approach, useful for estimates at large scales, requires extensive data about the system (David et al., 2001; Groffman et al., 2006). Some of this information is also based on assumptions that are not necessarily fulfilled, for example, that there are no significant sites of nitrogen storage in a system under study (Groffman et al., 2006). This method also does not provide much information on where or when denitrification is occurring (Groffman et al., 2006). Natural abundance stable isotopes do not measure actual denitrification rates, and are affected by many of the same assumptions as mass balance approaches (Groffman et al., 2006).

Groffman et al. (2006) conclude that despite some shortcomings, the acetylene-block method is one of the few available that allows for estimates over a wide scale, such as our study, and provides the best means for comparisons. Furthermore, this method allows ease of manipulations for examination of environmental denitrification regulators. Since the primary purpose of the

present study is to compare denitrification activity across a watershed, and to understand environmental regulators of the process, the chloramphenicol-amended acetylene block method was deemed the most appropriate.

2.0 STUDY INTRODUCTION

The Watershed Evaluation of Beneficial Management Practices (WEBs) program examines the water quality protection performance and economic viability of several agricultural BMPs across nine Canadian watersheds (Agriculture and Agri-Food Canada, 2010b). One practice in the STCW of southern Manitoba, Canada is the installation of a network of 50 small headwater reservoirs to aid in the prevention of flooding, erosion, and sedimentation. In addition to successfully providing these benefits, the reservoirs have also been shown to reduce nutrient loads downstream (Tiessen et al., 2011; Liu et al., 2014). With the proposal of new future reservoirs to be constructed in the greater Tobacco Creek Model Watershed (TCMW; Les McEwan and Don Cruikshank, personal communication), full understanding of reservoir nutrient processes is important to support ongoing efforts to improve water quality here. Furthermore, the TCMW is upstream of Lake Winnipeg, a large eutrophic lake. There is great interest in reducing nutrient loads into this system (Lake Winnipeg Stewardship Board, 2006; Environment Canada and Manitoba Water Stewardship, 2011).

To date, research on nutrient retention in STCW reservoirs has been limited to measuring nutrients upstream and downstream of the structures. Although this research has certainly yielded useful information, there is currently inadequate knowledge of nutrient processes involved. As a result, we are uncertain of the permanence of nitrogen removal, as well as design constraints that could be addressed to optimize nitrogen removal. Denitrification has been shown to exhibit relatively high activity in reservoir structures, due to lengthy contact time between water and sediment, and the settling of fine sediments and particulate nutrients (Wall et al., 2005; David et al., 2006; Tiessen et al., 2011). Denitrification is suspected to play a large role in nitrate removal within the TCMW.

In order to understand the role of these reservoirs in removing excess nitrogen from the watershed, denitrification activity was characterized across the watershed. The relationships between several environmental parameters and denitrification activity were also examined. Denitrification activity and environmental parameters were compared between reservoirs and stream pools in the watershed to examine relative importance of reservoirs as sites of

denitrification activity. Finally, nitrate saturation status of denitrification was determined as an indicator of whether denitrifying bacteria at these sites were overwhelmed by nitrate loads.

2.1 Purpose and objectives

The purpose of this research is to characterize denitrification activity and identify environmental parameters associated with denitrification in the Tobacco Creek Model Watershed to determine the role of reservoirs and stream pools in nitrogen removal.

Objectives:

1. Compare denitrification in reservoirs and stream pools

   Q1. Is denitrification significantly different between reservoirs and stream pools?

   H_0: Denitrification will not differ significantly between site types.

   Ha: Denitrification will differ significantly between site types.

2. Determine if nitrate saturation is occurring within the watershed

   Q1. Is nitrate saturation occurring in the watershed?

   H_0: There will be no cases of nitrate saturation across the watershed.

   Ha: Nitrate saturation will occur within the watershed.

   Q2. Is there a significant difference between nitrate saturation in reservoirs vs stream pools?

   H_0: There will be no significant differences in nitrate saturation status between site types.

   Ha: Nitrogen saturation status will differ between site types.

3. Determine important environmental parameters affecting denitrification

   Q1. Can water chemistry and sediment characteristics be used to predict denitrification rates?

   H_0: Water chemistry and sediment characteristics will not be related to denitrification and cannot be used to predict denitrification rates in the watershed

   Ha: Water chemistry and sediment characteristics such as sediment particle size, organic carbon content, water column temperature, pH, dissolved oxygen, ammonia, nitrate, soluble reactive phosphorus, total phosphorus,

specific conductance, and alkalinity can be used in a model to predict denitrification rates in the watershed

3.0 METHODS

3.1 Study area

3.1.1 Watershed location and characteristics

The TCMW, including its sub-watershed of South Tobacco Creek (~7,638 ha), is located approximately 150 km southwest of Winnipeg, Manitoba in the upper reaches of the Red River Basin (Hope et al., 2002). Land surrounding the TCMW (100,000 ha) is predominantly under cultivation, with approximately 83% in annual crops (TCMW, 2004 Water Strategy Blueprint document). Cultivated soils are mainly dark gray chernozems of loam or clay loam formed on calcareous glacial till of mixed shale, limestone, and granite over shale bedrock (Hope et al., 2002; Tiessen et al., 2011).

Agricultural settlement initiated much clearing of shrubs and forest, and drainage of wetlands, causing flooding, erosion and sedimentation (Hope et al., 2002; Glozier et al., 2006). These issues are exacerbated by the physiography of the region, particularly the sloping escarpment that causes a drop of 60 meters in elevation over 3 km (Tiessen et al., 2011), and clay soils that are relics of ancient glacial Lake Agassiz (TCMW, 2004 Water Strategy Blueprint document). Much of the watershed experiences poor drainage due to a combination of these features (TCMW, 2004 Water Strategy Blueprint document). Catchment headwaters drain from the Manitoba Escarpment uplands, and streams undergo heavy channelization just before reaching the Morris River. Here the water continues downstream to the Red River (Glozier et al., 2006). The low grade of the plains in the downstream reaches means that many streams do not have continuous channels to the Red River. Instead, streams pool in marshes and swamps below the escarpment (TCMW, 2004 Water Strategy Blueprint document). The watershed also has many streams referred to as temporary or episodic streams (Buttle et al., 2012). These streams experience greatly reduced flow or no flow at all, often flowing only in response to rainfall and snowmelt events.

The study area is classified as Aspen Parkland Ecoregion, and straddles the Boreal Transition and Lake Manitoba Plain Ecoregions (Glozier et al., 2006). The climate is semi-arid, with potential evapotranspiration exceeding actual precipitation. There are great seasonal variations in temperature and precipitation, which vary also with differing terrain across the watershed (Hope

et al., 2002). Mean annual precipitation varies from 590 mm in the uplands to 500 mm below the escarpment, with 25% falling as snowfall in the winter months (Hope et al., 2002).

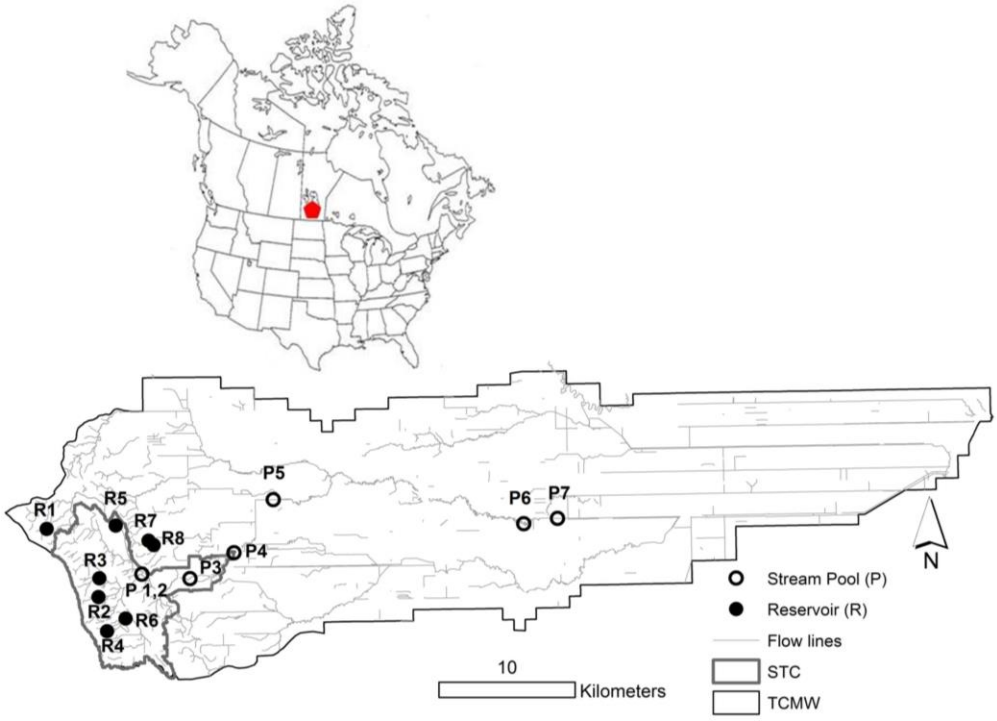

Figure 3.1. Location of sites within the Tobacco Creek Model Watershed (TCMW) and its sub-watershed of South Tobacco Creek (STC)

3.1.2 Beneficial management practices and monitoring history

Between 1985 and 1996, local landowners under the Deerwood Soil and Water Management Association (DSWMA) constructed 50 small-scale headwater dams in the TCMW to address flooding, erosion, and sedimentation issues (Hope et al., 2002). Of these dams, 26 lie within the STCW. A study by Tiessen et al. (2011) showed that dams successfully reduced peak flow, sediment, and nutrient loads. Three different dam types have been installed: dry dams, built to decrease peak flows by retaining water for a short time; backflood dams that retain water at a shallow depth over large cropland acreages before releasing; and multi-purpose dams which reduce peak flows, but retain 50% of the capacity for seasonal agricultural, recreational, or environmental purposes.

Land management, water flow, and water quality data have been collected from landowners in the region through the DSWMA since 1991 (Hope et al., 2002), and Environment Canada's hydrological monitoring stations (Environment Canada, 2011). In 2004, the STCW was added as a site under the Watershed Evaluation of Beneficial Management Practices (WEBs) program: a collaboration of government, academic, and local groups that assess environmental and economic performance of selected BMPs at sites across Canada (Agriculture and Agri-Food Canada, 2012). The greater TCMW strives to be a 'living watershed laboratory' through collaboration of farmers, communities, agriculture industry, government policy and decision makers, and scientific researchers (TCMW, 2004 Water Strategy Blueprint document). This unique level of landowner involvement and collaboration, along with extensive research and monitoring, has left an excellent legacy of data and local expertise on which to draw for this research.

3.1.3 Site selection

This study involves seven reservoirs created by DSWMA installed headwater dams in the STCW, one reservoir created by an earthen road dam, and seven stream pools in the STCW and greater TCMW. Sites were chosen based on accessibility, likelihood of not drying in late summer, and geographic spread across the watershed. Sites were selected from the western, highly dammed headwater regions, through the beginning of the eastern channelized region in order to gain good watershed representation. Stream pools that were expected to dry up in late summer were avoided to ensure sampling could continue at all sites throughout the study. Chosen stream pools were flowing in May and June. By July and August, however, some stream pools experienced reduced flow, or ceased flowing altogether, becoming stagnant ponds. Sites are coded from west to east and grouped into reservoirs and stream pools, such that the westernmost reservoir is R1, and the easternmost stream pool is P7 (Table 3.1).

Table 3.1. Site details

Code	Coordinates	Reservoir Type	Reservoir Area (km^2)	Additional Information
R1	49° 23' 45" N 98° 26' 21.7" W	Multi-purpose	No information	
R2	49° 21' 2" N 98° 23' 15" W	Multi-purpose	0.008	Cattle direct access
R3	49° 21' 47" N 98° 23' 12" W	Dry	0.030	Monitored †
R4	49° 19' 40" N 98° 22' 45" W	NA	0.004	Road dam, groundwater fed
R5	49° 23' 52" N 98° 22' 11" W	Multi-purpose	0.013	Drop outlet broken, may be functioning as dry dam
R6	49° 20' 10" N 98° 21' 38" W	Multi-purpose	0.023	Monitored †
R7	49° 23' 15" N 98° 20' 11" W	Dry	No information	
R8	49° 23' 4" N 98° 19' 53" W	Dry	No information	
P1	49° 21’ 55" N 98° 20' 37" W	NA	NA	Reduced flow in August
P2	49° 21' 55" N 98° 20' 37" W	NA	NA	Monitored ††
P3	49° 21' 44" N 98° 17' 42" W	NA	NA	
P4	49° 22' 44" N 98° 15' 0" W	NA	NA	Monitored †††, pooled in July and August
P5	49° 24' 50" N 98° 12' 35" W	NA	NA	Channelized area
P6	49° 23' 45" N 97° 57' 23" W	NA	NA	Reduced flow in July, pooled in August
P7	49° 23' 57" N 97° 55' 20" W	NA	NA	Channelized area, reduced flow in August

† Post-reservoir runoff monitoring and water sampling and analysis through the Watershed Evaluation of Beneficial Management Practices project

†† Environment Canada Hydrometric Gauging Station 05OF023

††† Environment Canada Hydrometric Gauging Station 05OF017

3.2 Water and sediment sampling

Water and sediment samples, as well as *in situ* measurements of water temperature, pH, dissolved oxygen, and specific conductance, were collected once in May, June, July, and August from each site. Water samples were collected for denitrification assays and alkalinity analysis by grab sampling 1 L of unfiltered water per site. *In situ* measurements were made using an Orion Star A329 Portable Meter (Thermo Scientific, Singapore). Water depth was measured with a meter stick. An additional 1 L of grab water sample was filtered through a combusted 0.7 µm

GF/F filter paper and collected using acid-washed, site-water rinsed bottles for NO_{3+} NO_2 (nitrate + nitrite) and DOC analyses. Approximately 15mL of filtered water was collected in acid-washed, site-water rinsed glass scintillation vials allowing for 10% headspace, preserved with 0.15 mL of 10% sulfuric acid, and frozen for ammonia analysis.

Sediment samples were collected from the top 5 cm to best capture the desired biological activity (Schaller et al., 2004; Inwood et al., 2005) using a handheld 4.5 cm diameter sediment corer, compositing approximately 10 cores along transects at each site. Samples were composited in order to obtain a range of characteristics across the width of the streams and away from the reservoirs' banks. Samples were transported on ice back to the laboratory to run assays as quickly as possible (1 - 5 days). Storage time sensitivity was determined in an experiment, where denitrification assays were run on local samples for 6 consecutive days with all other factors held equal (Appendix 9.1).

3.3 Laboratory analyses

3.3.1 Measuring denitrification

Sediment and water samples were analyzed for denitrification activity using the chloramphenicol-amended acetylene-block technique modified from Arango and Tank (2008). This technique uses acetylene to stop conversion of N_2O to nitrogen gas for easier measurement of the end product. Added chloramphenicol inhibits the synthesis of proteins, thus preventing new enzyme production in the assays (Smith and Tiedje, 1979; Inwood et al., 2005; Arango and Tank, 2008). Accumulated N_2O can then be measured over time to determine denitrification activity.

The chloramphenicol-amended acetylene-block technique has been slightly modified from Arango and Tank (2008) to reflect improvements in temperature manipulation, shaking and purging techniques. Slurries were created by adding 50 mL of unfiltered site water with chloramphenicol at a concentration of 6.0 mM to 25 mL of sediment subsamples for each site (Inwood et al., 2005). Each site had eight slurries: two controls, three acetylene-treated and three nitrate-amended. Nitrate-amended slurries were created with the addition of potassium nitrate (KNO_3) to ensure the availability of 100 mg NO_3-N per kg of sediment (Groffman et al., 1999), and were included in the study to test for nitrate saturation. Each slurry was sealed in a glass

media bottle with a septum cap and purged with nitrogen gas via a manifold for five minutes to induce anoxic conditions. Bottles were gently swirled after each minute to encourage mixing and ensure full purging.

Controls did not receive any acetylene (C_2H_2), and were used to quantify background N_2O production. Background N_2O concentrations amounted to 2% of N_2O production in reservoir samples, and 3% in stream pool samples. We did not correct for this amount in our calculations.

After purging, 15 mL of a mixture of 50% C_2H_2 + 50% N_2 gas was injected into the normal and nitrate-amended bottles to bring samples above atmospheric pressure, limiting the risk of contamination with O_2. Control bottles received 15 mL of 100% N_2 gas. Bottles were shaken to encourage mixing, and the time after injection was recorded. After 20 minutes, 10 mL headspace samples were withdrawn using a 10 mL syringe with 23G1 needle (PrecisionGlide by Becton Dickinson & Co, New Jersey), and injected into labeled 6 mL evacuated glass Exetainer vials. To return the bottles to pressure, 10 mL of N_2 was added to controls, and 10 mL of 10% C_2H_2 + 90% N_2 to the normal and nitrate-amended bottles.

Bottles were then placed on a VWR DS-500 Orbital Shaker (Henry Troemner LLC, New Jersey) in a temperature controlled room set to average ambient water temperature for the date sampled. The shaker table was set at 175 rpm, and the samples were left in the dark. For the first two sampling events (May and June), headspace samples were withdrawn and replaced with gas as described above. The second sample was taken after 70 mins, and every 60 minutes after that. Immediately after sampling and replacement, incubation and shaking resumed. After 3.5 hours (four gas samples), the procedure was stopped, and the water was removed from the bottles via a Pasteur pipette. Sediments were dried at 105°C until constant mass was reached, and dried sediment mass was determined via a Balance Classic Plus PB1502-S/FACT (Mettler Toledo, Switzerland).

The 3.5 hour incubation was determined to be longer than necessary. Time points were changed for the remaining sampling events (July and August). The procedure was the same as described above, but samples were taken every 30 minutes from the first sampling, for 2.5 hours (five gas samples). This change allowed the earlier and more linear portion of the experiment (more representative of natural rates) to be better captured.

Headspace samples were analyzed for N_2O concentrations with a Scion 456-Gas Chromatograph (Bruker, the Netherlands; Electron Capture Detector [injector temperature 60°C, column temperature 60°C, detector temperature 350°C, with an Ar carrier gas at 10 mL/min]). Denitrification activity was calculated using the accumulation of N_2O in the water and headspace, corrected for sample removal, along with the dry sediment mass and assay duration (Appendix 9.2). Areal estimates for comparison purposes were obtained according to standard soil methods given by Boone et al. (1999), using bulk density of the sediment, and a sampling depth of 5cm.

3.3.2 Water chemistry and sediment analyses

Methods for water and sediment analyses are indicated in Table 3.2. In brief, DOC was measured in triplicate via oxidative combustion-infrared analysis. Nitrate was measured in duplicate via automated colorimetry with azo dye following the reduction of nitrate by a copperized cadmium redactor. Note that this measurement includes the typically small amount of nitrite present in water, and is therefore reported here as nitrate + nitrite. Ammonia was measured in duplicate via the indophenol blue method after neutralizing the acidified samples with NaOH. Soluble Reactive Phosphorus (SRP) and Total Phosphorus (TP) were measured via the molybdate blue method, and alkalinity was measured via automatic titration. Sediment organic carbon was measured by loss on ignition, and median particle size was measured in triplicate by laser diffraction.

Table 3.2. Water chemistry and sediment analyses methods

Analysis	Sample Preparation	Method	Equipment	Citation
Water Chemistry				
Dissolved Organic Carbon (DOC)	Filtered at collection with combusted 0.7 µm GF/F filters	Non-Purgeable Organic Carbon analysis	Shimadzu TOC-V_{CPN}	Shimadzu Corporation, 2003
Nitrate + Nitrite (NO_3 + NO_2)	Filtered as above	Method NO3-002-A	Unity Scientific SmartChem 170 Discrete Analyzer	Unity Scientific, 2011a
Ammonia (NH_3)	Filtered as above, Preserved with 15 mL of 10% sulfuric acid, and frozen until analysis	Method AMM-002-A	Unity Scientific SmartChem 170 Discrete Analyzer	Unity Scientific, 2011b
Soluble Reactive Phosphorus (SRP)	Filtered at collection with 0.45 µm cellulose acetate filters	Molybdate blue method	UV-1601PC UV-Visible spectrophotometer	Murphy and Riley, 1962
Total Phosphorus (TP)	Digested in 3% potassium persulfate for 45 min at 121°C	Molybdate blue method	UV-1601PC UV-Visible spectrophotometer	Murphy and Riley, 1962; Menzel and Corwin, 1965; Wetzel and Likens, 1991
Alkalinity	Filtered in laboratory with combusted 0.7 µm GF/F filters	Automatic titration	TitraLab ABU52 Biburette	
Sediment Analyses				
Sediment Organic Carbon (SOC)	Dried to constant mass at 105°C, Sieved to omit >2mm particles	Loss on ignition : 400°C overnight	Muffle furnace	Schumacher, 2002
Mean Particle Size	Sieved as above, Air dried	Laser diffraction : Sonication = 90 seconds	Horiba LA-950 Particle Size Analyzer	Horiba Instruments, Inc., 2012

3.4 Statistical analyses

Denitrification activity data were determined to be non-normal via a Shapiro-Wilk test ($W = 0.60$, $p < 0.001$) and visual inspection of a quantile-quantile plot. Non-normality of denitrification data is often seen across the literature, commonly more closely resembling a lognormal distribution (Tiedje et al., 1989; Inwood et al., 2005; Arango & Tank, 2008). Denitrification data in the present study, however, could not be transformed to meet the assumptions of parametric statistical tests. Non-parametric alternative tests were used as described below, and run on RStudio software version 0.98.945 (The R Foundation for Statistical Computing), with significance determined at $\alpha = 0.05$ (95% confidence) unless otherwise stated. The mean of laboratory replicates for each sampling event within each site was used, so that mean values of all measured parameters were obtained for each month. When testing or comparing across months and sites however, median values were used to better approximate normal data. The coefficient of variation amongst laboratory replicates for acetylene-treated samples was 38%, while that for nitrate-amended samples was 21%.

3.4.1 Comparing denitrification activity across sites

Wilcoxon-Mann-Whitney Rank Sum Permutation Tests were used to determine if there were significant differences in denitrification activity between reservoirs and stream pools, and also to test for significant differences across months of the study. Permutation tests and their uses are described in detail in Kabacoff (2011). These tests are appropriate in cases where data comes from an unknown distribution. Essentially, the method performs the traditional statistic (Wilcoxon Rank-Sum Test in this case) for every permutation of randomly sorted data scores, uses the resulting test statistics to create an empirical distribution, and then tests whether the true test statistic of the original data lies within this distribution. Outputs of this statistic are reported as the Z test statistic, and P-value. A traditional Wilcoxon Rank-Sum Test was used to compare denitrification between early (May and June) and late (July and August) summer. Outputs of this statistic are reported as the W test statistic, and P-value.

3.4.2 Nitrate saturation

Nitrate saturation was determined by assessing whether there were significant differences in denitrification activity between acetylene-treated and nitrate-amended treatments. Sites were

identified as either nitrate-saturated (no difference) or nitrate-unsaturated (significantly different). Student's T-tests were used to determine the nitrate saturation status of each individual site in each month, as non-parametric alternatives, which relied on ranks, failed due to low sample size ($n \leq 7$). Outputs of this statistic are reported as the T test statistic, and P-value. A Pearson's Chi-squared test was used to determine whether nitrate saturation status differed between reservoirs and stream pools. Outputs of this statistic are reported as the X^2 test statistic, and P-value. A bar chart was used to visualize the comparison of nitrate saturation status between reservoirs and stream pools in each month, with standard error calculated according to proportion estimates with pooled variance:

$$\sqrt{\hat{p}(1-\hat{p})\ x\ ({n_1}^{-1} + {n_2}^{-1})}$$

Where

$\hat{p} = (X_1 + X_2)\ x\ (n_1^{-1} + n_2^{-1})$

X = proportion of saturation

n = number of sampling cases

3.4.3 Predictors of denitrification

Wilcoxon-Mann-Whitney Rank Sum Permutation Tests (described in section 3.4.1.) were used to determine if there were significant differences in parameters between site types, and between site types in early vs late summer. This method was also used to test for significant differences in parameters correlated to denitrification that varied over the study period.

Kendall's Rank Correlation Tests were applied to all parameters of interest to determine if they were correlated with denitrification activity. Outputs of this statistic are reported as the T test statistic, tau correlation estimate, and P-value. Regression tree analyses were run to determine the parameters important in determining denitrification activity. Classification tree analyses were run to determine the parameters important in determining nitrate saturation status. Trees explain variation in a response variable by splitting data into groups based on thresholds of explanatory variables, and displaying the results graphically. The tree is constructed by splitting the data according to selected criteria that maximizes homogeneity in each group. The trees in this study

were split with the criteria that a node (point of partition) had to have at least five data points to consider further splitting. Failure to further split resulted in a terminal node. Trees were pruned in order to minimize the cross-validated relative error while maintaining a satisfactory explanation of the data. De'ath & Fabricius, (2000) provide an overview on classification and regression trees and their use in ecological research.

3.4.4 Geographical and catchment influences

The reservoirs were intentionally installed predominantly in the headwaters of the Tobacco Creek, creating a geographically unbalanced study design when comparing reservoirs and stream pools. Given that sites within the STCW may experience different conditions than sites outside the STCW due to factors of land use, elevation, and soil type (Hope et al., 2002; TCMW, 2004 Water Strategy Blueprint document), we tested for relationships between longitude and environmental parameters, including denitrification activity. Linear regressions were used to determine whether any parameters varied with longitude (approximately upstream/downstream) across the watershed. Outputs of this statistic are reported as the R^2 correlation, and P-value. A Wilcoxon Rank-Sum Test was used to test whether any parameters differed from sites within the STCW compared to sites outside the STCW (TCMW).

4.0 RESULTS

4.1 Denitrification activity

There was a high degree of variation in denitrification activity in the present study. The lowest denitrification activity value found during the study was in the stream pool P1 during June, at 9.8 x 10^{-8} mg N g DM (dry matter)$^{-1}$ h^{-1}. Eight of the 10 lowest denitrification activity values were found within stream pools. The highest denitrification activity value found was in the reservoir site R5 during May, at 1.1 x 10^{-3} mg N g DM^{-1} h^{-1}. All 10 of the 10 highest denitrification activity values were found within reservoir sites. When median denitrification activities from all 15 sites were ranked from lowest to highest, the lower end of the data was dominated by stream pools, and the higher end by reservoirs, (Table 4.1).

Table 4.1. Sites ranked from lowest to highest median annual denitrification activity

Site	Denitrification Activity (mg N g DM^{-1} h^{-1})
P1	2.1 x 10^{-7}
P3	3.2 x 10^{-7}
P2	1.1 x 10^{-6}
P4	1.8 x 10^{-6}
R1	2.1 x 10^{-6}
P7	1.2 x 10^{-5}
P5	1.7 x 10^{-5}
R6	2.9 x 10^{-5}
P6	3.9 x 10^{-5}
R8	1.4 x 10^{-4}
R3	1.6 x 10^{-4}
R7	1.9 x 10^{-4}
R5	2.5 x 10^{-4}
R2	3.1 x 10^{-4}
R4	4.2 x 10^{-4}

Reservoirs were shown to have significantly higher denitrification activity than stream pools ($P = <0.01$, Table 4.2). This difference was driven by significant differences in May and June. In July and August denitrification activity did not differ significantly between reservoirs and stream pools (Table 4.2, Figure 4.1). Denitrification activity across the watershed was higher in May and June than in July and August ($W = 574$, $p < 0.01$). This trend was driven, however, by

changes within the reservoirs alone, as there was no significant difference in denitrification activity between early and late summer across the stream pools (W = 76, p = 0.96).

Table 4.2. Results of permutation tests comparing median denitrification activity between reservoirs and stream pools in each month of sampling

Month	**Median Denitrification Activity for Reservoirs (mg N g DM^{-1} h^{-1})**	**Median Denitrification Activity for Stream Pools (mg N g DM^{-1} h^{-1})**	**Z**
May	1.7×10^{-4}	1.9×10^{-6}	3.0**
June	4.6×10^{-4}	3.2×10^{-7}	2.5*
July	3.2×10^{-6}	2.3×10^{-6}	0.5
August	6.1×10^{-7}	1.9×10^{-6}	0.8
All Months	1.8×10^{-4}	1.8×10^{-6}	2.8**

**Significant at p<0.05*

*** Significant at p<0.01*

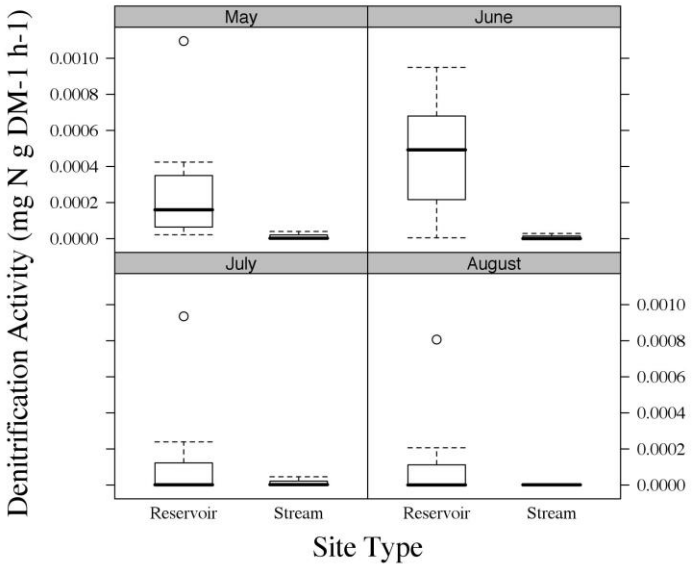

Figure 4.1. Denitrification activity in reservoirs and stream pools by month. Boxes represent the middle 50% of data, with a bold line indicating the median value. Dashed lines are the whiskers, which indicate the remaining 50% of data. The flat dashed lines show the maximum and minimum values, excluding any outliers, which are shown as open circles

4.2 Nitrate saturation

There was widespread evidence of nitrate-saturation across our study sites, and over time, with 49% of measurements showing nitrate-saturation. Nitrate saturation occurred in each month of the study in both reservoirs and stream pools. Only one of 15 sites did not exhibit nitrate saturation at any point in the study (R1). There were no sites that exhibited nitrate saturation throughout the entire study.

Reservoirs were generally nitrate-unsaturated during the study period (on average, 59% of measurements showed no saturation). This trend was largely due to low frequency of nitrate saturation in July and August, whereas in May and June, reservoirs exhibited higher incidences of saturation (Figure 4.2). Stream pools were generally nitrate saturated (on average, 60% of measurements showed saturation), with higher occurrences in June and July. Pearson Chi-squared results did not show a significant difference between the occurrence of nitrate saturation in reservoirs and stream pools ($X^2 = 2.1$, $p=0.15$).

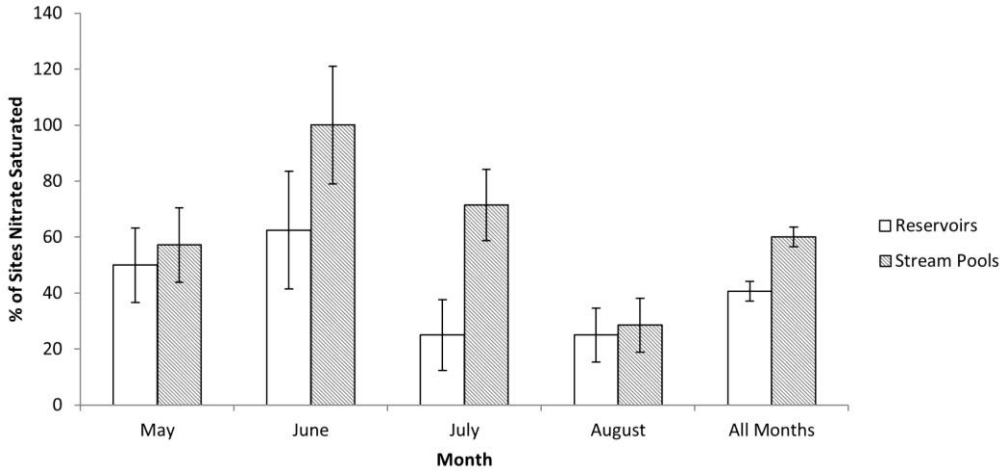

Figure 4.2. Percent of sites that were nitrate-saturated for each month of the study, with error bars indicating standard error of proportions with pooled variance

4.3 Predictors of denitrification

Environmental parameters were highly variable during the study (Table 4.3). For example, pH varied from 6.7 – 9.5, dissolved oxygen from 0.8 to 14.3 mg L^{-1}, specific conductance from

261.0 to 1425.0 µS cm^{-1}, and median particle size from 5.1 to 390.0 µm values. Sediment organic carbon was found to be significantly higher in reservoirs (Table 4.4). Parameters significantly higher in stream pools included pH, dissolved oxygen, and specific conductance (Table 4.4).

Table 4.3. Minimum and maximum values of environmental parameters measured across all sites in study considering all months

Parameter	Range
Water Temperature (°C)	7.1 – 25.5
pH	6.7 – 9.5
Dissolved Oxygen (mg L^{-1})	0.8 – 14.3
Specific Conductance (µS cm^{-1})	261.0 – 1425.0
Dissolved Organic Carbon (mg L^{-1})	5.1 – 15.0
NO_{3+} NO_2 (Nitrate + Nitrite) (mg N L^{-1})	<0.1 – 5.4
NH_3 (mg N L^{-1})	0.1 – 2.0
Soluble Reactive Phosphorus (mg L^{-1})	<0.1 – 0.5
Total Phosphorus (mg L^{-1})	<0.1 – 1.0
Alkalinity (µg mL^{-1})	73.3 – 359.0
Sediment Organic Carbon (%)	0.4 – 4.6
Median Particle Size (µm)	5.1 – 390.0

Table 4.4. Comparison of annual median values for environmental parameters between reservoirs and stream pools. Asterisks denote whether median values were significantly different between reservoirs and stream pools according to a permutation test

Parameter	Reservoirs	Stream Pools
Water Temperature (°C)	16.5	16.8
Water Depth at Sampling (cm)	16.5	18.5
pH	7.7 *	8.1 *
Dissolved Oxygen (mg L^{-1})	7.7 *	9.2 *
Specific Conductance (µS cm^{-1})	494.8 *	686.0 *
Dissolved Organic Carbon (mg L^{-1})	9.9	8.2
NO_{3+} NO_2 (mg N L^{-1})	1.4	0.1
NH_3 (mg N L^{-1})	0.5	0.4
Soluble Reactive Phosphorus (mg L^{-1})	0.2	0.1
Total Phosphorus (mg L^{-1})	0.3	0.2
Alkalinity (µg mL^{-1})	236.9	256.1
Sediment Organic Carbon (%)	2.4 ***	0.7 ***
Median Particle Size (µm)	50.4	233.7

*Significant at $\alpha = 0.05$

***Significant at $\alpha = 0.001$

All environmental parameters of interest (Table 4.4) were tested for correlation with denitrification activity. The variables found to be correlated are shown in Table 4.5. Nitrate + nitrite showed the strongest positive correlation, followed by SOC. The strongest negative correlation was with median particle size, followed by pH. Of the environmental parameters found to correlate with denitrification activity, only $NO_3 + NO_2$ was found to vary significantly between months, as determined by a permutation test (Table 4.6). $NO_3 + NO_2$ was higher in early summer, peaking in June. Dissolved oxygen was also higher in early summer, peaking in May. Water temperature was highest in July, and lowest in June. Alkalinity was higher in late summer, peaking in August. Specific conductance was highest in July, and lowest in June. Ammonia was higher in late summer, peaking in August.

Table 4.5. Environmental parameters determined to be correlated with denitrification activity using a Kendall's Rank Correlation Test

Parameter	**T statistic**	**tau**	**P-value**
$NO_3 + NO_2$	82	+0.56	<0.01
Sediment Organic Carbon	81	+0.54	<0.01
Median Particle Size	28	-0.47	<0.05
pH	32	-0.39	<0.05

Table 4.6. Environmental parameters found to vary significantly over time using a permutation test

Parameter	**May**	**June**	**July**	**August**	**P-value**
$NO_3 + NO_2$ (mg L^{-1})	0.7	1.7	<0.1	<0.1	<0.001
Dissolved Oxygen (mg L^{-1})	10.0	9.3	7.3	8.7	<0.001
Water Temperature (°C)	14.5	12.8	23.4	16.8	<0.001
Alkalinity (µg mL^{-1})	205.3	176.4	286.5	305.7	<0.001
Specific Conductance (µS mL^{-1})	521.0	355.0	728.0	624.0	<0.01
NH_3 (mg L^{-1})	0.1	0.3	0.4	0.8	<0.01

Of the parameters that differed between reservoirs and stream pools, only specific conductance differed over the whole study period. Overall differences in dissolved oxygen between the two site types was driven by significant differences in late summer only, while early summer saw no significant differences between the two site types ($Z = 0.5$, $p = 0.64$).

Regression trees were used as an additional means of detecting influential environmental parameters related to denitrification activity (Figure 4.3). Across all sites, the tree showed $NO_3 +$

NO_2 as the primary node, with SOC as the secondary node at high $NO_3 + NO_2$ concentrations. The tree for reservoir sites showed the same parameters as that of all sites taken together. Across stream pools, the tree showed median particle size as the primary node, with $NO_3 + NO_2$ as a secondary node at low particle sizes.

Classification trees were used to detect the most important environmental parameters affecting nitrate saturation (Figure 4.4). Across all sites, the tree showed $NO_3 + NO_2$ as the only node. The tree for reservoir sites also showed $NO_3 + NO_2$ as the only node. Across stream pools, the tree showed total phosphorus as the primary node, with sediment organic carbon as the secondary node at low total phosphorus concentrations. At low denitrification activity ($<6.49 \times 10^{-7}$ mg N g DM^{-1} h^{-1}; the first quartile of denitrification data), only median particle size was identified in association with saturation. Denitrification activity was only observed to be this low in stream pools. Classification trees were validated with data obtained from stream sites in a watershed approximately 200 km northwest of the study area. By using the criteria in the stream pool classification tree, only 44% of the sites were successfully sorted into the terminal nodes (Appendix 9.3). When the criterion for all sites was used, however, the sorting success rose to 78%. The same success rate of 78% was seen if the criterion for low denitrification was used.

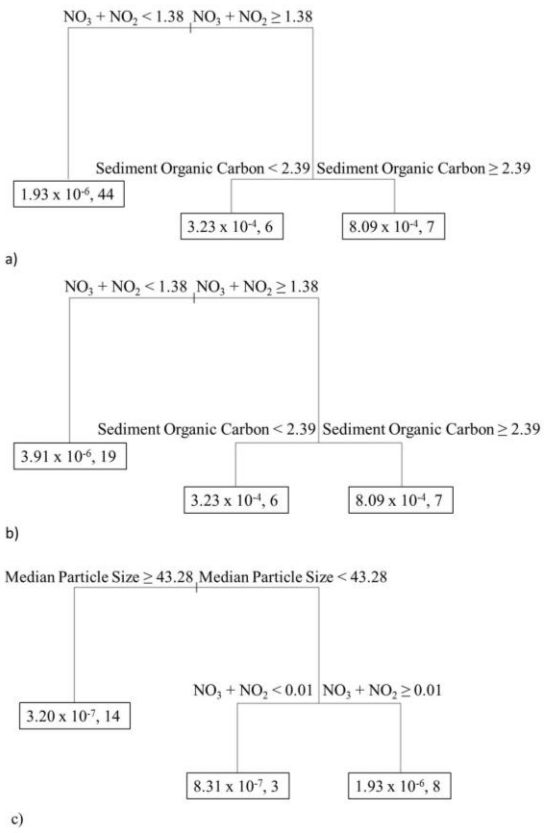

Figure 4.3. Regression trees, showing the environmental parameters with the greatest influence on denitrification activity across a) all sites (cross validated relative error = 0.73 ± 0.28; re-substitution relative error =5.59 x 10^{-8}), b) reservoirs (cross validated relative error = 1.03 ± 0.31; re-substitution relative error =1.13 x 10^{-7}) and c) stream pools (cross validated relative error = 1.3 ± 0.40; re-substitution relative error =2.68 x 10^{-10}). The leaves, or terminal nodes, indicate median denitrification activity followed by the number of data points in the node. Denitrification activity is reported in mg N g DM^{-1} h^{-1}, $NO_3 + NO_2$ in mg N L^{-1}, sediment organic carbon in %, and median particle size in µm. The height of the vertical lines represents the proportion of total sums of squares explained by each split.

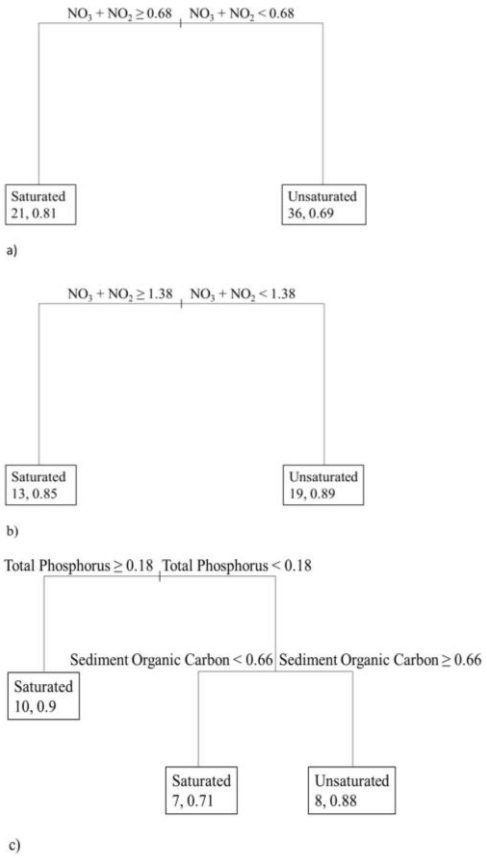

Figure 4.4. Classification trees, showing the environmental parameters with the greatest influence on nitrate saturation across a) all sites (cross validated relative error = 0.71 ± 0.13; re-substitution relative error =0.35), b) reservoirs (cross validated relative error = 0.69 ± 0.2; re-substitution relative error =0.28), and c) stream pools (cross validated relative error = 1.6 ± 0.24; re-substitution relative error =0.64). The leaves, or terminal nodes, indicate nitrate saturation status by the number of data points in the node, and the proportion of those points that fulfil the given saturation condition. Nitrate + nitrite is reported in mg N L^{-1}, total phosphorus in mg $L^{-1}$and sediment organic carbon in percent dry weight. The height of the vertical lines represents the proportion of total sums of squares explained by each split.

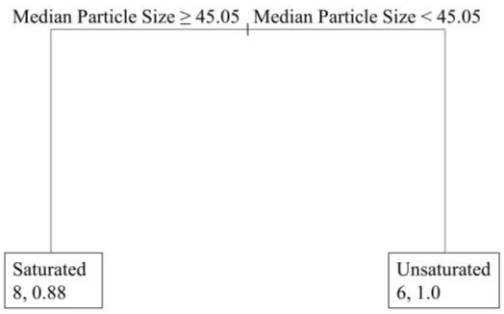

Figure 4.5. Classification tree showing the environmental parameters with the greatest influence on nitrate saturation when denitrification was low (<6.49 x 10^{-7} mg N g DM^{-1} h^{-1}) across all sites (cross validated relative error = 0.43 ± 0.22; re-substitution relative error =0.2). The leaves, or terminal nodes, indicate nitrate saturation status by the number of data points in the node, and the proportion of those points that fulfill the given saturation condition. Nitrate + nitrite is reported in mg N L^{-1} and median particle size in µm. The height of the vertical lines represents the proportion of total sums of squares explained by each split.

4.4 Geographical and catchment influences

Denitrification activity did not vary with longitude when all sites were taken together, but annual median data from stream pools showed a negligible trend towards higher activity in the eastern (downstream) reaches (R^2 = 0.48, p = 0.05). When all sites were considered, SRP was found to be higher in the western reaches (upstream) of the watershed (R^2 = 0.34, p < 0.05), and specific conductance was found to be higher in the eastern reaches (downstream) (R^2 = 0.84, p < 0.001). When stream pools were examined alone, several parameters differed across the geographical gradient (Table 4.7).

Table 4.7. Environmental parameters that differed across the geographical gradient within stream pools, as determined via linear regression. Annual median values were used

Environmental Parameter	Directionality of higher values	R^2, P-value
Specific Conductance (µS cm^{-1})	East (downstream)	0.99, < 0.001
Soluble Reactive Phosphorus (mg L^{-1})	West (upstream)	0.96, < 0.001
Dissolved Organic Carbon (mg L^{-1})	East (downstream)	0.66, < 0.05
Ammonia (mg N L^{-1})	East (downstream)	0.60, < 0.05
Alkalinity (µg L^{-1})	East (downstream)	0.58, < 0.05
Denitrification Activity (mg N g DM^{-1} h^{-1})	East (downstream)	0.48, 0.05

Outside of the STCW, specific conductance was higher in the eastern reaches ($R^2 = 0.78$, $p < 0.05$), and median particle size ($R^2 = 0.60$, $p < 0.05$) was higher in the western reaches. Sediment organic carbon was negligibly higher in the western reaches ($R^2 = 0.56$, $p = 0.05$). No other variables were explained by longitude. Within the STCW, specific conductance was higher in the eastern reaches ($R^2 = 0.78$, $p < 0.001$). NH_3 ($R^2 = 0.45$, $p < 0.05$), SOC ($R^2 = 0.37$, $p < 0.05$), and median particle size ($R^2 = 0.85$, $p < 0.001$) were higher in the western reaches ($R^2 = 0.43$, $p < 0.05$). Denitrification activity did not vary by longitude in either watershed. In addition, the only significant differences in environmental parameters found between sites inside and outside the STCW were specific conductance, which was higher outside the STCW ($W = 45$, $p < 0.05$), SRP, which was higher inside the STCW ($W=6$, $p < 0.05$), and median particle size, which was higher inside the STCW ($W = 10$, $p < 0.05$). There was no significant difference in denitrification between sites inside and outside of the STCW.

5.0 DISCUSSION

5.1 Denitrification activity

Denitrification activity in this watershed was lower than reported in other agricultural watersheds (Table 5.1). This result is likely a consequence of lower nitrate concentrations and SOC in this catchment. Differences in conditions across studies can be caused by multitudes of factors; from agricultural practices to climatic and hydrological regimes. The numerous BMPs implemented in the STCW may be one of the reasons for the lower denitrification activity we saw in our study, as successful BMPs can reduce nitrate loading to surface water (Agriculture and Agri-Food Canada, 2010b).

Table 5.1. Comparison of denitrification rates and environmental parameters across selected studies with similar methods and land uses. Empty values signify that the parameter was not measured in the study or was measured in an incomparable unit. Mean values are reported, unless otherwise stated. Source 1 = downstream (agricultural) values from Kemp and Dodds, 2002a; 2 = Schaller et al., 2004; 3 = agricultural values from Inwood et al., 2005; 4 = agricultural values from Arango and Tank, 2008; 5 = Herrman et al., 2008; 6 = this study.

Parameter	**Values**					
Source	1	2	3	4	5	6
Denitrification Activity ($\mu g\ N\ g\ DM^{-1}\ h^{-1}$)	-	0.8	0.2	1.1	<0.1 – 28.0 †	<0.1 ††
Areal Denitrification Activity ($mg\ N\ m^{-2}\ d^{-1}$)	0.4	-	-	-	-	23.3††
pH	7.5	8.3	-	7.9	8.0	8.0††
Dissolved Oxygen ($mg\ L^{-1}$)	10.4	11.8	-	8.4	-	9.0††
Specific Conductance ($\mu S\ cm^{-1}$)	-	582.0	-	-	-	591.0††
Dissolved Organic Carbon ($mg\ L^{-1}$)	-	3.3	9.2	7.0	3.5††	9.8††
Nitrate ($mg\ N\ L^{-1}$)	0.6	6.6	4.4	12.2	9.3††	0.3††
Ammonium ($mg\ N\ L^{-1}$)	<0.1	-	0.2	0.2	-	0.4††
Soluble Reactive Phosphorus ($mg\ L^{-1}$)	-	-	0.1	0.2	-	0.1††
Sediment Organic Carbon (%)	-	-	2.6	3.5	-	1.3††

† AFDM (Ash free dry matter) – sample has been combusted

†† Median values reported

The median denitrification activity in our study was higher for reservoirs, at 1.8×10^{-4} mg N g $DM^{-1}\ h^{-1}$ compared to 1.8×10^{-6} mg N g $DM^{-1}\ h^{-1}$ amongst stream pools. One difference between stream pools and reservoirs which likely contributed to the difference in denitrification activity among these site types is the higher concentrations of SOC in reservoirs. Sediment organic

carbon was shown to be positively correlated with denitrification, consistent with the importance of organic carbon in fuelling denitrification (Knowles, 1982). A second factor differing between these site types was the higher oxygen concentrations present in stream pools, which would be expected to inhibit denitrification rates (Knowles, 1982), although this factor was not identified as a significant correlate in this study. The final factor shown to differ between reservoirs and stream pools was pH, with lower pH in reservoirs than in stream pools. In this study, pH was shown to be negatively correlated with denitrification activity. This negative correlation is in contrast to many studies that have found the relationship to be positive (Cleemput & Patrick, 1974; Muller et al., 1980; Baeseman et. al, 2006), and may be explained by a negative correlation between SOC and pH (-0.47, $p < 0.05$), and a positive correlation between dissolved oxygen and pH (0.39, $p < 0.05$). However, Martin et al. (2001) also found a negative relationship between pH and denitrification in their study, concluding that if nitrate is present in high concentrations, low pH may not be inhibitory for denitrification. Additionally, our lowest pH values were in the range of 6.7, hardly below the oft-cited ideal range of 7 – 8 (Bryan & Delwiche, 1976; Knowles, 1982).

The effect of SOC, pH, dissolved oxygen, and other parameters on denitrification has been described in detail by Knowles (1982). Not discussed in detail, however, is the effect of sediment particle size on denitrification. We expected sediment to be finer in the reservoirs, due to lower flow allowing settling of fine particles (Bergamaschi et al., 1997; Wall et al., 2005) as opposed to stream pool habitats. These stream habitats can be subject to significant scouring under high flow conditions, leading to export of fine sediment particles and associated nutrients. Fine sediments have been shown to have low porosity, creating anoxic conditions that favour denitrification (García-Ruiz et al., 1998; Solomon et al., 2009). Higher rates of sedimentation also facilitate the sedimentation of particulate-bound nutrients, which are more readily held by fine sediments (Brady & Weil, 2008). We expected to see differences in particle size between site types contributing to effects on denitrification. While the expected negative correlation between denitrification and particle size was shown, we did not see a statistically significant difference in median particle sizes found in reservoirs and stream pools.

The wide range of median particle sizes found in stream pools (5.0 – 390.0 µm; fine silt to fine sand according to USDA 2014) contributed to a large variance that makes comparisons difficult.

Median particle size in reservoirs ranged from 18.0 – 63.0 µm, which corresponds to fine silts on the lower end to very fine sands on the higher end. There were two stream pool sites (P4 and P7) with very fine particle sizes (5.0 – 9.0 µm), both of which experienced minimal flow in the later months of the study. P4 was sampled just downstream of a culvert under a road, where fine sediments may accumulate after being washed through the culvert. P7 is a channelized stream portion with vegetation in banks throughout the streams, which may have helped to stabilize the sediment. This type of variation suggests that these site types may have more heterogenous sediment dynamics and flow characteristics than anticipated. Nonetheless, these characteristics may help explain the higher concentration of SOC in reservoirs, which, along with DOC in the water column, serve as electron donors that fuel denitrification (Knowles, 1982).

Only two reservoirs exhibited annual median denitrification activity lower than some of the stream pools. The more striking case, R1, is a reservoir situated on the north branch of the Tobacco Creek. This site is surrounded by trees and vegetation, which may explain why it had the highest single measurement of SOC in the study (4.6% in August). The site had $NO_3 + NO_2$ concentrations that were < 10% of the median concentration for reservoirs, more closely matching the concentrations seen in stream pools. Unlike the stream pools, however, R1 had SOC values more than three times that of the average for stream pools. Low $NO_3 + NO_2$ concentrations coupled with moderate to high SOC may be the reason that R1 was the only site in the study in which nitrate saturation was never observed. Although denitrification activity was low at this site, further examination of R1 could lend important insights into the efficient removal of nitrate, as several other sites in the watershed exhibiting low denitrification rates also experienced nitrate saturation.

5.2 Geographical and catchment influences

While reservoirs and stream pools differed in several parameters, it is difficult to tease apart the effects of their geographical location. Reservoirs are located in the headwaters of the watershed, with five of eight reservoirs located in STCW. The stream pools are all located downstream of the reservoirs, to the east, with only two stream pools on a comparable longitude to the reservoirs. Four of the seven stream pools are within the STCW. Further complicating the distribution is the presence of the escarpment, which changes the hydrological regime, sediment composition, and other factors within the watershed.

Examination of factors that differed across the watershed between reservoirs and stream pools suggest that reservoirs generally had conditions more favourable for denitrification, regardless of geographical influences. No parameters that showed a significant correlation with denitrification were found to vary across the longitudinal gradient when examining both site types across the entire study area. Median particle size was higher, however, in the western reaches of both the South Tobacco Creek sub basin (n=9) and of sites external to the STCW (n=6). Sediment organic carbon was also higher in the western reaches within the STCW. Despite gradients in these correlates, denitrification activity was not found to vary. In addition, despite significant differences between median particle size at sites inside and outside of the STCW, denitrification activity did not vary between the basins. Although it is difficult to properly assess due to the spread of reservoirs compared to stream pools, these results suggest that longitudinal gradient and differences between sub basins of the TCMW likely do not have a significant confounding influence on our assessment of denitrification activity in the reservoirs and stream pools of the watershed. From what we have observed from correlations with denitrification, most gradients found across the watershed (with the exception of SOC in the STCM basin) actually present favourable conditions to the stream pools in the east. These findings provide evidence that the presence of characteristics associated with denitrification is a factor of the site types themselves (reservoirs and stream pools) and not just the result of geographical variance across the watershed.

5.3 Predictors of denitrification

Our findings of environmental parameters that correlated to denitrification activity tended to agree with those found in the literature, especially for agriculturally-influenced landscapes. The most oft-cited factor limiting denitrification is nitrate availability (Kemp and Dodds, 2002b; Inwood et al., 2005; Herrman et al., 2008), which was also the most strongly correlated variable in the present study, and the primary influence in our regression tree for all sites. Other influences on denitrification were the stimulating effect of organic carbon (Arango and Tank, 2008; Inwood et al., 2005), and temperature (García-Ruiz et al., 1998; Inwood et al., 2005; Herrman et al., 2008), and the inhibiting effect of oxygen (Kemp and Dodds, 2002b). Sediment particle size also plays a role in denitrification in the literature, with finer sediments favouring denitrification activity (García-Ruiz et al., 1998), as was also found in the present study.

The present study also found organic carbon to be important, specifically that found in the sediment. This finding illustrates the need to examine both dissolved and sediment organic carbon as energy sources for denitrification, unlike work that has focused on DOC alone (Holmes et al., 1996; Inwood et al., 2007). Although we did not test the relative importance of the two carbon sources through addition experiments in the laboratory, some inferences can be made from what was observed by comparing values across sites. Only SOC was correlated with denitrification activity, suggesting that this source is the more important of the two forms in our study. This finding is in agreement with Inwood et al. (2005) and Herrman et al. (2008), who concluded that sufficient SOC rendered DOC unimportant as a control on denitrification rates. Dissolved organic carbon is not always available to denitrifying bacteria due to differences in its lability (Fork & Heffernan, 2014) and potential competition with overlying heterotrophic bacteria (Bernhardt & Likens, 2002; Johnson et al., 2012). These findings raise interesting questions of how organic carbon inputs could be managed in reservoirs and stream pools to provide more favourable conditions for denitrification.

5.4 Nitrate saturation

Nitrate saturation reflects an environmental limit on nitrate removal capacity, which can be imposed by carbon limitation, temperature, or other factors. It also tends to reflect poor ecological status – that is, this indicator identifies the times and locations where nitrate concentrations are sufficiently high that they are saturating an important ecosystem service. Although denitrification can be a substantial nitrogen sink in aquatic environments (Galloway et al. 2003), when nitrate concentrations are high, nitrate export is substantial. Mulholland et al. (2009) maintain that in order to achieve high denitrification efficiency, total nitrogen concentrations should not exceed 1 mg N L^{-1}. This threshold seems to fit our data fairly accurately, at least in terms of dissolved inorganic nitrogen, which is assumed to be the most dominant nitrogen pool (Tiessen et al., 2011). There were only three instances where nitrate was not saturating in a stream pool site with dissolved inorganic nitrogen ($NO_3 + NO_2 + NH_3$) over 1 mg N L^{-1}. Reservoirs showed higher capacity to increase denitrification activity, with seven cases where saturation was not observed at high dissolved inorganic nitrogen concentrations (>1 mg L^{-1}).

We may refine our threshold using a classification tree for saturation. An examination of dissolved inorganic nitrogen concentrations reveals that 2.33 mg N L^{-1} is a more accurate threshold for all sites taken together for our data (Appendix 9.4; 92% of cases are nitrate saturated above this level). This threshold was more successful at describing reservoirs. When stream pools alone were examined, the threshold fell to 0.87 N L^{-1} with 60% of cases saturated above this level. This threshold is similar to the one reported for a reservoir in Illinois by Wall et al. (2005). Although heavy nitrate loading can overwhelm denitrification, it seems that attributes of reservoirs in our study such as higher SOC, and lower dissolved oxygen, may delay this saturation to higher concentrations of nitrogen.

July was the only month in which there was some evidence of a significant difference in nitrate saturation status between reservoirs and stream pools. This finding suggests that in July, reservoirs had greater capacity for increased denitrification. This could possibly be due to the higher supplies of organic carbon found in reservoirs throughout the season. As temperatures rose in July, we could expect enhanced metabolic processes by well-fueled denitrifiers, allowing them to assimilate added nitrate. Higher oxygen concentrations and lower SOC in the stream pools (Table 4.6) may have limited the ability of denitrifiers to take advantage of these warmer conditions and added nitrate.

Given that nitrate saturation can be used as an indicator of ecosystem health, based on the system's ability to retain or remove excess nitrogen in the system (Kemp and Dodds, 2002b; Earl et al., 2006), our results suggest that the proportion of nitrate saturation during the study (49% of measurements) is indicative of a fairly poor capacity to cope with nitrogen loading in the TCMW. While the preferred solution is to prevent excessive nitrogen loading (Lewis et al., 2011), there are also possibilities of environmental manipulation, such as carbon inoculation, to increase microbial activity in order to retain nutrients. Experimentation with carbon inoculation has had some success in reducing nitrate-contamination of groundwater (Matějů et al., 1992; Bucco et al., 2014) and in phosphorus retention of degraded urban streams (Aldridge et al., 2009). In order to implement these actions in a system, limiting factors for the process of interest (in this case, denitrification) must be identified, and suitable conditions for the microbes can then be provided to allow increased rates of the process, leading to desirable outcomes (in this case, nitrogen removal).

Management activities, such as beneficial management practices, can directly impact nitrate, but also affect factors that influence denitrification, such as dissolved organic matter. For example, leaf litter addition or riparian restoration may enhance organic carbon availability (McGlynn & McDonnell, 2003; Hook & Yeakley, 2005; Aldridge et al., 2009) and in doing so, support increased denitrification. While watershed-scale organic carbon management may be feasible, Stanley et al. (2012) recommend that attempts to manage organic carbon start with small local changes, which could include activities like managing riparian areas of reservoirs to enhance litter inputs. Vidon et al. (2010) reviews some of the potential of environmental modifications in removing nutrients and other contaminants, noting that conditions favourable to addressing one process may conflict with other processes (such as phosphorus retention, organic matter mineralization, and pesticide degradation). Modifying conditions does not come without risks, and requires reliable information on limiting parameters, nutrient transport, and potential contaminant interactions in the system of interest. This caution is true of the BMP studied here, where possible tradeoffs between ecosystem services of nitrogen retention, and disservices, such as emission of greenhouse gases may merit consideration.

We have already discussed some controls over nitrate saturation across reservoir and stream pool sites, but perhaps the more interesting cases were those in which nitrate saturation occurred under low denitrification activity, suggesting poor conditions for nitrate removal via denitrification. Only stream pools exhibited denitrification activity in the lowest quartile of our data. Median particle size was the only parameter that emerged as important under these low denitrification activity, with saturation occurring when particle size was greater than 45.05 µm (coarse silt according to USDA classification). It is also interesting that in stream pool sites, NO_3 + NO_2 was not an important factor in our nitrate saturation models. In this saturation model, TP was the primary node, with saturation occurring in 90% of cases where TP was greater than 0.18 mg L^{-1}, although the reason for this separation is not clear.

Data collected from stream sites within Manitoba, but external to our watershed, was used to validate classification trees, with varying success. The model for stream pools, thought to be most likely to describe the validation sites, did a poor job predicting nitrate saturation (44%). Greater success was seen when the model for all sites was used (78%), although the criterion (NO_2 + NO_3 < or > 0.68 mg L^{-1}) did not split the data, as all sites had very low values and only

two were found to be saturated. Perhaps the model describing sites exhibiting low denitrification (which were all stream pools in the present study) is the most appropriate, and these data could be sorted with a success of 78% on the basis of median particle size. These findings suggest that the TCMW is more susceptible to nitrate saturation than other watersheds in Manitoba, due in part to higher NO_2 + NO_3 concentrations.

5.5 Effects of hydrological events and water residence time

Differing site characteristics may help explain differences in the capacities of the sites to react to hydrological events, which occurred to a much higher degree in the early summer. Peak snowmelt occurred in late April 2013, causing high runoff through early May. Two large rainfall events in mid and late May brought approximately 70 mm and 65 mm of water in the span of two days per event (Environment Canada, 2014). This snowmelt and rainfall is likely responsible for the higher concentrations of NO_3 + NO_2 seen in early summer ($Z=4.5$, $p = < 0.001$), as runoff could be expected to carry dissolved and sediment bound nutrients from surrounding agricultural land. The fact that significant differences in denitrification activity between reservoirs and stream pools occurred in early summer alone suggests important relationships between hydrological events and denitrification activity may exist. For example, denitrifying bacteria in the reservoirs may have been able to take advantage of higher nitrate during events, supporting higher denitrification activity in May and June. Stream pools, however, did not show heightened denitrification activity during this time, possibly due to other factors limiting denitrification, such as higher oxygen, or lower SOC.

In periods of high flow, reservoirs in the area are designed to retain excess water and release it in a slow and controlled manner to avoid flooding downstream. This retention affords reservoirs further opportunity to undergo denitrification, as there is greater contact time between the nitrate in the water and the bacteria in the sediment. Denitrification activity in the reservoirs seemed to respond well to nutrient influxes after snowmelt and rain events, in that rates were significantly higher than that of stream pools. Past work in this watershed, however, highlights the potential for flow-through dynamics to develop in reservoirs at extreme high flows, contributing to high rates of nitrate export (Tiessen et al., 2011), and limiting the role of denitrification as a nitrogen retention mechanism during these important periods. Residence time, therefore, will be an important determinant of the extent of nitrate removal via denitrification.

All stream pool sites experienced high flow during the snowmelt and rainfall events in May and June, allowing for very little contact time between the sediments and water. This short residence time of water likely resulted in a higher proportion of nitrate being exported rather than denitrified, due to reduced time for interaction with the microbes (Pinay & Naiman, 2002; Seitzinger et al., 2006; Powers et al., 2012). Interestingly, there was no significant difference between denitrification activity in the stream pools in these early months compared to later months of the study when flow was slower, water temperatures were warmer, and oxygen was lower. This finding suggests that the lower denitrification activity found in stream pools throughout the duration of the study may be due to other characteristics of the sites. Flow regimes affect many of these site characteristics, including sedimentation of nutrients, and distribution of particle size. The importance of flow could be further explored by examining differences between continuously flowing stream sites and temporary stream sites.

Temporary streams tend to be poorly studied: Buttle et al. (2012) points to the lack of information on temporary streams, identifying that these important and abundant hydrological sites provide conditions suitable to biotic communities. In fact, temporary streams, due to their decreased flows, can possess characteristics more similar to reservoirs. Manis et al. (2014) found that when flowing, an ephemeral stream had higher organic matter, nitrate, DOC, and denitrification activity than its permanent counterpart. Although differences found in Manis et al. (2014) are undoubtedly influenced by the fact that the temporary stream in question existed due to tile drainage, and thus likely had high nutrient concentrations, it still illustrates the need to consider these temporary streams in the nitrate removal process. Many stream pool sites in the present study undergo intermittent flow, hence contribute to this understudied ecosystem type.

5.6 Limitations and further research

Denitrification activity was estimated by the chloramphenicol-amended acetylene block technique which, like any other method, has drawbacks. This method can underestimate activity due to inhibition of the nitrate-supplying nitrification process via acetylene (Hynes and Knowles, 1978; Walter et al., 1979). Seitzinger et al. (2006) report that the role of the oft-coupled nitrification process in supplying nitrate to denitrification is likely only significant at nitrate concentrations less than 0.3 mg L^{-1}, which occurred in nearly half of our measurements (n=28).

Concentrations of $NO_3 + NO_2$ were less than 0.3 mg N L^{-1} in all but two sites (R4 and R5) for at least one month. In 32% of these cases, nitrate saturation was detected, suggesting that any nitrate supplied by nitrification would not increase denitrification activity. It is possible that in the remaining cases (n=19), nitrification was also contributing to the nitrate pool for denitrification, but could not be observed in the lab due to inhibition of the process via acetylene. This means that we could be underestimating the denitrification activity in these cases. Cases with low $NO_3 + NO_2$ occurred more frequently in reservoirs (63% of cases), and in late summer (89% of cases), suggesting that denitrification at these times and places is most likely to be underestimated. Due to the relatively high concentrations of NH_3 found in our study (Table 5.1), nitrification should be examined to better understand the sources contributing to denitrification activity in the watershed.

There are important limitations in translating laboratory-based denitrification results to understanding of *in-situ* rates of nitrate removal. One important limitation is our inability to characterize the endpoints of this process *in situ*. Of particular interest is the proportion of dinitrogen (N_2) vs. N_2O, which is a potent greenhouse gas. The production of N_2O during denitrification raises questions regarding environmental tradeoffs, specifically whether this ecosystem service of nitrate removal is worth the N_2O emissions. Several studies have examined the factors that contribute to higher proportions of N_2O production. Mulholland et al. (2009) found that in 49 streams studied, N_2 far exceeded N_2O (94.3 – 99.9 % of production). Nevertheless, several studies have found that the proportion of N_2O produced increases with available nitrate concentrations (Hendzel et al., 2005; Silvennoinen et al., 2008; Baulch et al., 2011) suggesting that some of our sites may be producing significant amounts of N_2O. However, the availability of organic carbon has been shown to decrease proportions of N_2O produced (Weier et al., 1993; Hendzel et al., 2005), suggesting that high carbon availability found in many of our sites may aid in the reduction of N_2O. Further study on this matter could reveal interesting information on controls of N_2O and whether the productive reservoirs are releasing significant greenhouse gases as they remove nitrate.

Denitrification activity in our study was estimated over a fairly short sampling season, excluding other parts of the year in which denitrification could also be playing a role. Much of the watershed, however, ceases to flow from late summer to spring, giving little scope for a

comparative analysis in other seasons. Interesting information, however, could be obtained on under-ice denitrification activity and environmental conditions in select sites over the winter (e.g. (Baulch et al., 2011). This information could aid in understanding what conditions are optimal for nitrogen removal in winter and during snowmelt to help optimize BMP design. These insights would be of particular interest, given that snowmelt is the dominant period of nutrient runoff, but provides conditions that seem to limit denitrification. Extremely high water level and flow during snowmelt and rainfall events prohibited us from sampling during this very interesting time, and caused the exclusion of three sites from our data in June. Perhaps other methods or protocols could be employed to access samples at these times to learn more about the effect of hydrological events on the capacity for nitrate removal in the watershed. Measuring changes in denitrification activity during these events could lend very interesting information about the capacity of the microbes to continue denitrifying under high flow conditions, provide insights on the importance of sediment-derived versus water-column-derived nutrients for the process, and help support watershed-scale modeling of nitrogen retention processes.

As with many research studies, our study would have benefited from a greater sampling size, either by sampling more broadly across space, across more field seasons, or during winter. Some important relationships may have been lost, especially when seasonal medians were used, due to low sample size and the necessity of using nonparametric statistics. This concern especially applies to nitrate saturation, as it appears that reservoirs should be less likely to be nitrate-saturated than stream pools at least transiently (Figure 4.2). Additional representation of sites from the sub basins of the Tobacco Creek Model Watershed would also be beneficial for comparison purposes, as well as increased study of both permanently and temporarily flowing stream sites.

Overall, this study provides an overview of characteristics that differ between reservoirs and stream pools of the Tobacco Creek Model Watershed, which supplies an excellent foundation for additional research to better understand nitrate removal in the watershed. While we cannot calculate the exact proportion of nitrate retained in these BMPs, rough estimates indicate that in R6, up to 46.6 kg N could be removed via denitrification over the summer months, representing about 60% of the total nitrogen retention reported to be retained by the reservoir (Tiessen et al., 2011; Appendix 9.5). As this reservoir was one of the lower ranking reservoirs in terms of

denitrification activity, we can appreciate that nitrate removal in the reservoirs of the watershed is fairly substantial. This work demonstrates that constructed reservoirs have habitat well-suited to permanent nitrogen removal via denitrification, identifies conditions associated with increased denitrification, and shows that nitrate concentrations in reservoirs and stream pools are frequently in excess of ecological thresholds, resulting in limited nitrate attenuation.

6.0 STUDY CONCLUSIONS

Reservoirs in the TCMW exhibited higher denitrification activity than stream pools, and were generally not nitrate-saturated during the study, meaning that they had the capacity to take up further nitrate. Water residence time likely plays a role in shaping conditions favourable to denitrification in the reservoirs. Pooled water allows higher sedimentation of organic carbon that fuels denitrification, encourages buildup of fine particle sizes that exclude oxygen and hold nutrients (Knowles 1982; Bergamaschi et al., 1997) and increases water-sediment contact time for microbial interaction with dissolved nutrients (Seitzinger et al., 2006). The ability of reservoirs to retain water may have been particularly important in the early summer (May and June) when snowmelt and two large rain events occurred. The influx of nutrients that these events undoubtedly swept into the waterways from surrounding agricultural land may have allowed an increase in denitrification activity in the reservoirs that explains the significantly higher activity than that seen in stream pools.

Despite the higher capacity for denitrification in the reservoirs, the watershed as a whole experienced considerable nitrate-saturation (49% of cases were saturated), and further efforts to reduce nitrate loading to the watershed are warranted. Knowing the conditions which encourage and hinder the denitrification process is important for the utilization of beneficial management practices that aim to reduce nitrate loading and other environmental stressors in the Tobacco Creek Model Watershed. Our results illustrated that $NO_3 + NO_2$, SOC, median particle size, and pH were all significantly correlated with denitrification, and that pH and SOC differed significantly between reservoirs and stream pools. These differences could be contributing to the difference in denitrification activity found between these two habitat types. The strongest correlate of denitrification rates, and primary node in most classification trees predicting saturation, however, was $NO_3 + NO_2$.

Findings from this study can direct management in the design and location of additional reservoirs in the watershed which are being proposed for the near future (Les McEwan and Don Cruikshank, personal communications) to maximize conditions favourable to denitrification activity. In particular, riparian vegetation increases the availability of organic carbon for denitrifying bacteria, and the build-up of fine sediment particles aids the process by retaining nutrients and creating anaerobic conditions (García-Ruiz et al., 1998; Solomon et al., 2009).

These and other factors can be manipulated in current reservoirs, or taken into account in the installation of new ones by locating reservoirs in well-vegetated areas with geomorphic conditions favourable to fine sediment deposition.

Of course, there are a host of factors to keep in mind, and great care should be taken to ensure that modifying reservoir design in favour of denitrification does not have adverse effects on other ecosystem services, or undesired tradeoffs in terms of agricultural productivity. For example, to increase denitrification by maximizing contact time between sediments and the water column, a large, shallow reservoir would achieve the most success. A reservoir with these dimensions, however, would have numerous implications. First if a goal of reservoir construction is flood control, a shallow reservoir may fail to contain sufficient volumes of water. Additionally, we must consider the land that would need to be allocated to make way for a large reservoir, with associated loss of cropland, grazing land, or wildlife habitat. Costs of installation and maintenance are important considerations, as different dimensions of the reservoir and associated outflow control mechanisms may change costs. In particular, due to reservoirs acting as sediment traps, a shallow reservoir may fill up quickly, requiring maintenance to dredge out excess sediment, or shortening the lifetime of the reservoir altogether. Dredging sediment would result in the loss of good habitat for denitrifying bacteria, as well as stores of carbon sources to fuel the denitrification process. Finally, installation of new reservoirs may create long-term issues when they reach their engineered lifespan, a current concern in the management of larger reservoirs (Poff & Hart, 2002; Stanley & Doyle, 2003).

In general, reservoir management must strike a balance to: maximize flood control; provide high water residence time that maximizes sediment to water contact, provide sufficient carbon stores for denitrification; minimize cost; and minimize diverted crop and grazing land. Reservoirs must attain all of this while ensuring that other key ecosystem services are not lost, such as provision of habitat for wildlife, and the retention of other nutrients of concern, such as phosphorus. Wildlife habitat can be preserved by leaving vegetated riparian zones at reservoir sites, and avoiding installation of reservoirs in areas of high wildlife use and diversity. Phosphorus, the primary limiting nutrient in freshwater systems (Schindler, 1974) can also be retained in reservoir structures (Tiessen et al., 2011; Liu et al., 2014). Understanding the controls of

phosphorus retention in the reservoirs of the TCMW will allow for the design of reservoirs that function to retain both nitrogen and phosphorus and will result in better watershed health.

Optimizing nutrient retention in agricultural watersheds may also involve channel design, which is commonly performed for other objectives in this region, such as drainage (TCMW, 2004 Water Strategy Blueprint document). Consideration of design attributes that facilitate higher denitrification activity may help overcome the nitrate saturation that was prevalent in stream pools. For example, meandering rather than channelized or straight streams will slow flow, encouraging the buildup of finer particles, and promoting conditions more favourable to denitrifying microbes.

This study focuses on abiotic environmental parameters that affect denitrification, but the responses to these parameters are controlled by complex characteristics of the microbial community. The process of denitrification is utilized by many groups of microbes (Zumft, 1997), and it is not possible to quickly estimate the unique identities and properties in a study of this nature. Molecular approaches to examining microbial communities can reveal information of the selection for, and conservation of denitrification genes, which demonstrates the importance of the process in the system under study (Tiedje et al., 1989). Combining information on denitrification rates in response to abiotic parameters, and microbial community structure and function can separate information on drivers of the process. Manis et al. (2014) found that changes in denitrification in response to hydrological changes was due to physiological shifts and not due to the abundance or composition of denitrifiers, whereas Baxter et al. (2012) found the abundance of the N_2O reductase genes to be the secondary controlling factor of denitrification. A study on the microbial community structure and function in the TCMW would complement the findings of abiotic controls on denitrification and give a better understanding of the importance of the process in this system.

Reservoirs of the TCMW are first and foremost flood control structures; a role we witnessed in action in May and June. Due to their success in this regard, as well as their capacity for nitrate removal, we recommend that a watershed-scale assessment of nitrogen loading and denitrification rates be performed, providing context for the contribution of nitrogen removal within the reservoirs. Small-scale reservoirs as seen in this watershed may be a good solution to help limit the effects of excess nitrate in similar watersheds with ephemeral streams, where fish

migration is not an issue, and in streams that are prone to flooding, leading to multiple benefits of this BMP.

7.0 GENERAL CONCLUSIONS

Intensified agriculture has shaped modern society, thanks to the supply of vast amounts of food to support city-dwelling non-producers. Unfortunately, with this increased intensification comes great stress on the world's land and water resources. Expansion of agricultural land has led to the draining of wetlands, the channelization of waterways, and the clearing of forests, and has caused increased erosion, habitat loss, and nutrient loading to water sources (Carpenter et al., 1998; Tilman et al., 2001; Montgomery, 2007).

Thankfully, there is growing movement to mitigate these issues, particularly through the use of BMPs. Successful BMPs must be effective at stopping the source of the problem (e.g. reducing nutrient inputs), or mitigating the effects of the problem (e.g. capturing nutrients in transport before they reach surface or groundwater). Beneficial management practices must also be economically effective, which is perhaps the largest barrier to overcome. We have the technology and knowledge to solve many of the issues with agricultural production today, but the solutions can be expensive, vary in efficacy for different regions, and incentives for implementing them can be low. Beneficial management practices can be quite costly to install and manage, and can also represent a loss in terms of displaced agricultural land (Agriculture and Agri-Food Canada, 2010a). Many agricultural producers cannot pass these increased costs to buyers, and so must have an altruistic attitude, or be otherwise compensated (Agriculture and Agri-Food Canada, 2010a).

Baumgart-Getz et al. (2012) found that the most important factors to adoption of BMPs were access to and quality of information, financial capability, and being connected to an agency or local network of farmers or watershed groups. Many agricultural producers are good stewards of the land who genuinely want to minimize their impact on the environment, but are constrained by economics and practicality. Producer-based agencies, such as Manitoba's DSWMA, are very successful in providing all three of Baumgart-Getz et al.'s (2012) adoption criteria. These agencies collaborate with government, universities, and other institutions to research solutions, fund expensive projects, and provide a local network of support.

To be successful, these groups must be established largely through the initiative of local landowners, have committed individuals as leaders, have well defined boundaries to achieve

realistic goals, and build strong partnerships with institutions that can offer logistical and financial support when needed (TCMW, 2004 Water Strategy Blueprint document). Researchers and decision makers who are interested in contributing to solutions for sustainable agriculture must engage landowners in the process. It is these collaborations that will allow us to solve the problem of feeding the world while preserving our planet.

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9.0 APPENDICES

9.1 Denitrification storage experiment

Introduction

Field sampling for a denitrification assay experiment occurred in the Tobacco Creek Model Watershed in southern Manitoba, a distance of approximately 800 km from the laboratory at the University of Saskatchewan in Saskatoon, Saskatchewan. Samples were shipped or driven to Saskatoon as soon as possible, as the denitrification analysis is known to be time sensitive. Nearly all studies of this nature try to analyze denitrification activity within 24 hours. Since this timeframe could not be accomplished due to the distance to the laboratory, a storage experiment was conducted to ensure that differences in transit and storage time to analysis did not confound denitrification assay results.

Methods

Water and sediment samples were collected from a temporary pond in an agricultural area near Saskatoon, Saskatchewan (52° 7'44.47"N, 106°33'9.71"W). Water samples were collected for denitrification assays by grab sampling unfiltered water. Sediment samples were collected from the top 5 cm using a handheld 4.5 cm diameter sediment corer, compositing approximately 10 cores along six transects. Samples were transported on ice back to the laboratory where one set was run immediately, and the remaining five sets were stored at 4°C to be run each day thereafter.

Sediment and water samples were analyzed for denitrification activity using the chloramphenicol-amended acetylene-block technique (Arango and Tank, 2008). Headspace samples were analyzed for nitrous oxide (N_2O) concentrations with a Scion 456-Gas Chromatograph. Denitrification activity was calculated using the accumulation of N_2O in the water and headspace, corrected for sample removal, along with the dry sediment mass and assay duration.

Denitrification activity values for each of the days were checked for normality via a Shapiro-Wilk Normality test, and averaged before applying a one-way ANOVA analysis and a linear regression analysis.

Results

Denitrification data was determined to be normal ($W = 0.96$; $p = 0.61$). Denitrification activity did not vary significantly over the course of the experiment ($F = 0.001$, $p = 0.98$). Date of analysis was not a significant explanatory variable of denitrification activity ($F = 0.001$, $p = 0.98$).

Discussion and conclusion

This storage experiment revealed that any changes in denitrification activity due to storage prior to analysis were not detectable over the course of six days. This finding is in agreement with Parkin et al. (1984), who found that soil samples could be stored up to 19 days with no significant impact on denitrification. An experiment by Wall et al. (2005) showed that sediment samples could be stored up to 24 hours with no effects on denitrification, but did not reveal whether longer storage times showed effects, or were even tested. These results suggest that denitrification may not be affected by storage times up to 6 days when using the chloramphenicol-amended acetylene block technique.

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9.2 Denitrification activity calculations

Step 1. Calculate moles of air in headspace of sample bottle using the ideal gas law, correcting for over pressuring of headspace (A)

{absolute pressure(atm) x headspace volume(L) x overpressure correction [volume inserted(L)/headspace volume(L)]} / [ideal gas constant(L x atm x K^{-1} x mol^{-1}) x absolute temperature (Kelvin)]

Step 2. Calculate moles of N_2O in headspace from known concentration (ppm) as measured in sample (B)

[A(mol) x sample concentration(ppm)] /1,000,000 ppm

Step 3. Calculate mg of N_2O-N in headspace (C)

B(mol) x molecular mass of N in N_20(28.02g x mol^{-1}) x [1000 mg x g^{-1}]

Step 4. Calculate the concentration (mol x L^{-1}) of N_2O in the aqueous phase, using Weiss and Price's (1980) solubility coefficient, the F value (D)

sample concentration (ppm) /{1,000,000ppm x F value(mol x L^{-1}x atm^{-1}) x overpressure correction[volume inserted(L)/headspace volume(L)]}

Step 5. Convert the concentration (M) of N_2O in the aqueous phase to moles (E)

D(mol x L^{-1}) x volume of water in sample(L)

Step 6. Calculate the mg of N_2O-N in the aqueous phase (F)

E(mol) x molecular mass of N in N_20(28.02g x mol^{-1}) x [1000 mg x g^{-1}]

Step 7. Add the mg of N_2O-N in the aqueous phase and gaseous phase (G)

F(mg) + C(mg)

Step 8. Calculate the mg N_2O-N removed during sampling (H)

B(mol) x [sampling volume(L) / headspace volume(L)] x *molecular mass of N in $N_2$0(28.02g x mol^{-1}) x [1000 mg x g^{-1}]*

Step 9. Calculate cumulative N_2O-N produced (I)

$$G(mg) + H(mg)$$

Step 10. Calculate the slope of the regression of cumulative N_2O-N production over assay time (J)

$$\sum(x - \bar{x})(y - \bar{y}) / \sum(x - \bar{x})^2$$

where x = time to sampling(h)

$\bar{x}$ *= mean time to sampling (h)*

y =I(mg)

$\bar{y}$ *=mean I(mg)*

Step 11. Calculate denitrification activity (g N_2O-N x mg DM^{-1} x h^{-1}), using sediment weight of each sample (K)

J(mg x h^{-1}) / sediment weight(g DM)

where DM = dry matter

Step 12. Calculate areal estimate for denitrification activity (mg N_2O-N x m^{-2} x h^{-1}), according to Boone et al., 1999. (L)

K(mg x g DM^{-1}x h^{-1}) x bulk density of sediment(g x cm^{-3}) x volume of sediment in 1000 cm^{-2} at sampling depth(cm^{-2} x cm) x non-rock fraction of sediment

References

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Weiss, R. F., & Price, B. A. (1980). Nitrous Oxide Solubility in Water and Seawater. *Marine Chemistry*, *8*, 347–359. doi:10.1016/0304-4203(80)90024-9

9.3 Classification tree validation

Introduction

Due to the highly variable nature of denitrification, findings of key environmental parameters associated with nitrate saturation may be restricted to small areas with little scope for extrapolation of results. Data from nine stream sites within a 75 km radius from Brandon, Manitoba (125 km west of study site) were used to validate the results from classification trees predicting nitrate-saturation.

Methods

Water and sediment samples were collected from 15 sites in the Tobacco Creek Model Watershed in Manitoba, as well as from 9 sites located throughout southern Manitoba. Water samples were collected for denitrification assays and alkalinity analysis by grab sampling unfiltered water. Sediment samples were collected from the top 5 cm using a handheld sediment corer, compositing approximately 10 cores along six transects. *In situ* measurements such as water temperature, pH, dissolved oxygen, and specific conductance were taken at all sites with an Orion Star A329 Portable Meter (Thermo Scientific). Additional grab water samples were filtered through a combusted 0.7 µm GF/F filter paper and collected using acid-washed, site-water rinsed bottles for NO_{3+} NO_2 (nitrate + nitrite) and dissolved organic carbon analyses. A portion of the filtered water was collected in acid-washed, site-water rinsed glass scintillation vials, preserved with sulfuric acid, and frozen for ammonia analysis.

Sediment and water samples were analyzed for denitrification activity using the chloramphenicol-amended acetylene-block technique (Arango and Tank, 2008). Some samples received additional nitrate to test for nitrate saturation. Headspace samples were analyzed for nitrous oxide (N_2O) concentrations with a Scion 456-Gas Chromatograph (Bruker). Denitrification activity was calculated using the accumulation of N_2O in the water and headspace, corrected for sample removal, along with the dry sediment mass and assay duration.

Classification trees were used to predict nitrate saturation for the sites in the Tobacco Creek Model Watershed (Figure A1). For each external site (Table 1), classification trees developed for all sites within the Tobacco Creek Model Watershed, for stream pools alone, and for sites with low denitrification (<6.49 x 10^{-7} mg N g DM^{-1} h^{-1}) were used as templates to sort the sites into one of the terminal nodes, on the basis of the given parameters and their thresholds. The saturation status at that node was then compared with the saturation status at that site.

Table A1. Locations of stream sites used to validate predictive denitrification model.

Site Name	**Coordinates**
Bosshill	49°50'5.94"N 100°57'47.23"W
Gopher	49°46'56.04"N 100°57'48.12"W
Bailey's	49°52'11.82"N 100°40'13.28"W
Brierwood	49°56'31.14"N 100°31'49.86"W
Hamiota	49°59'23.82"N 100°28'26.82"W
Lower Oak	50° 1'10.20"N 100°24'35.46"W
Elgin	49°34'9.12"N 100°14'7.92"W
Little Souris	49°43'57.72"N 99°50'53.58"W
Willow West	49°53'9.96"N 99°48'5.82"W

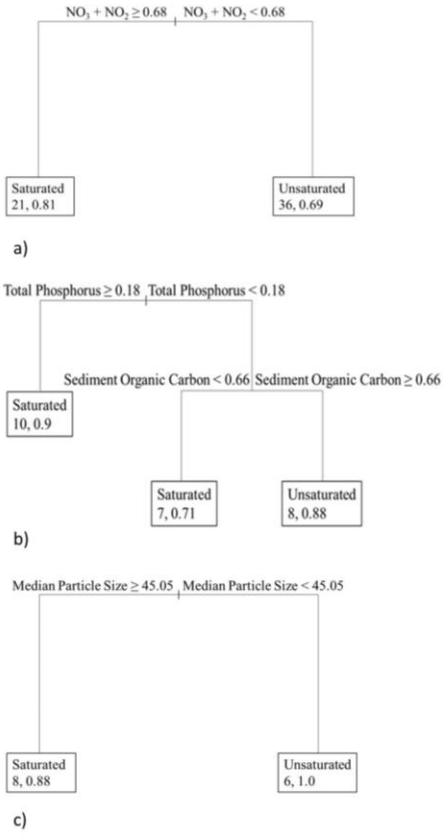

Figure A1. Classification trees as predictors of nitrate saturation to be validated with external data from southern Manitoba watersheds using a) all sites included in study, b) stream pools alone, and c) sites exhibiting low denitrification (<6.49 x 10^{-7} mg N g DM^{-1} h^{-1})

Results

Five of the nine sites were properly sorted (56%) using the stream pool classification tree. Seven of the nine sites were properly sorted (78%) using the all sites classification tree, and the low denitrification (<6.49 x 10^{-7} mg N g DM^{-1} h^{-1}) tree.

Discussion and conclusion

The stream model was not very successful in predicting nitrate saturation status using data from the stream survey when the stream pool classification tree was used. This success was much higher when the criterion for all sites was used, and when the criterion for low denitrification was used. This finding suggests that factors controlling nitrate saturation in streams may be quite specific to the Tobacco Creek Model Watershed and not necessarily applicable elsewhere. This

difference may be a factor of higher $NO_3 + NO_2$ concentrations in the TCMW compared to that of the sites external to the watershed, which had median $NO_3 + NO_2$ concentration of only 0.01 mg L^{-1}. The model for low denitrification is likely the most appropriate to use, as the splitting threshold for the all sites model was not reached by any of the validation sites.

9.4 Dissolved inorganic nitrogen thresholds

Introduction

Mulholland et al. (2009) assert that at nitrogen concentrations exceeding 1 mg L^{-1}, denitrification may be inefficient, leading to saturation of the process. We investigated thresholds of dissolved inorganic nitrogen, assumed to be the main contributing pool in our system, on nitrate saturation status to determine if Mulholland et al.'s (2009) threshold is true for our study.

Methods

Classification trees were developed using $NO_3 + NO_2 + NH_3$ data from all sites of interest, split on the basis of nitrate saturation status. One tree was created from all sites of interest, and another tree used data from stream pool sites alone (excluding reservoir habitats).

Results

The classification tree using all sites showed a threshold of 2.33 mg N L^{-1}, with 92% of measurements above this threshold experiencing nitrate saturation. The tree using stream pools showed a threshold of 0.87 mg N L^{-1}, with 60% of measurements above this threshold experiencing nitrate saturation.

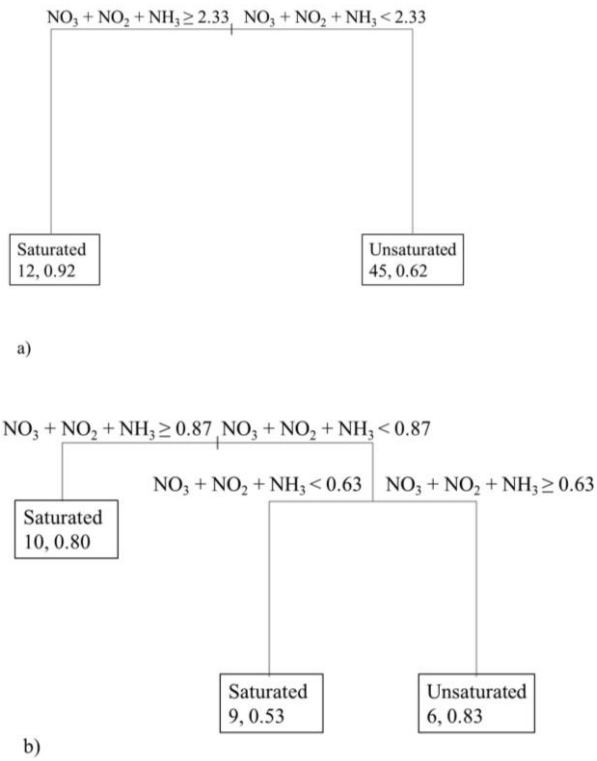

Figure A1. Classification trees, showing thresholds of nitrate + nitrite + ammonia ($NO_3 + NO_2 + NH_3$) associated with nitrate saturation of denitrification for a) all sites in the study (cross validated relative error = 0.68 ± 0.13; re-substitution relative error =0.33), and b) stream pools (cross validated relative error = 0.9 ± 0.2; re-substitution relative error =0.36). The leaves, or terminal nodes, indicate nitrate saturation status by the number of data points in the node, and the proportion of those points that fulfil the given saturation condition. Nitrate + nitrite + ammonia is reported in mg N L^{-1}. The height of the vertical lines represents the proportion of total sums of squares explained by each split.

Discussion and conclusion

The threshold for nitrogen concentrations determining nitrate saturation at our sites was higher than that reported by Mulholland et al. (2009) when all sites were examined, with a large influence from reservoirs that were less likely to be saturated. We saw a similar threshold with stream pools (0.87 mg N L^{-1}), although the proportion of saturation above this value was not very high (60%). These findings suggest that reservoirs are better able to remove nitrogen before being saturated.

References

Mulholland, P. J., Hall, Jr., R. O., Sobota, D. J., Dodds, W. K., Findlay, S. E. G., Grimm, N. B., Hamilton, S. K., McDowell, W. H., O'Brien, J. M., Tank, J. L. Ashkenas, L., R., Cooper, L. W., Dahm, C. N., Gregory, S. V., Johnson, S. L., Meyer, J. L., Peterson, B. J., Poole, G.C., Valett, H. M., Webster, J. R., Arango, C. P., Beaulieu, J. J., Bernot, M. J., Burgin, A. J., Crenshaw, C. L., Helton, A. M., Johnson, L. T., Niederlehner, B. R., Potter, J. D., Sheibley, R. W. & Thomas, S. M. (2009). Nitrate removal in stream ecosystems measured by 15N addition experiments : Denitrification. *Limnology and Oceanography*, *54*(3), 666–680.

9.5 Nitrogen removal estimates

Introduction

Installed reservoirs of the Tobacco Creek Model Watershed have been shown to exhibit increased rates of denitrification compared to other areas of the watershed. To estimate the actual amount of nitrogen removed in the reservoirs, an estimate for one well-studied reservoir was conducted.

Methods

Information from one reservoir in this study (R6) was gathered from this dissertation and from Tiessen et al. (2011) to calculate nitrogen removed.

Results

Areal denitrification estimate for R6 = 23.3 mg N m^{-2} d^{-1}

Area of R6 when full = 31,000 m^2 → Assume an average area during the summer of 20,000 m^2 (not full for entire summer)

Denitrification estimated at 23.3 mg N m^{-2} d^{-1} x 20,000 m^2 = 466,000 mg N d^{-1} = 0.466 kg N d^{-1}

If summer is ~100 days then denitrification takes care of 0.466 kg N d^{-1} x 100 d = **46.6 kg N (summer)**

Discussion and conclusion

Rough calculations of denitrification in the R6 site at Tobacco Creek Model Watershed estimate that 46.6 kg N may be removed in the summer months. The average reduction of total nitrogen in

the summer was calculated at 77 kg N by Tiessen et al. (2011), suggesting that denitrification may account for just over half of total nitrogen removal in the reservoir. Given that this reservoir exhibited one of the lower rates of denitrification in our study compared to the other reservoirs, denitrification could be playing a large role in nitrogen removal in the watershed.

References

Tiessen, K. H. D., Elliott, J. A., Stainton, M., Yarotski, J., Flaten, D. N., & Lobb, D. A. (2011). The effectiveness of small-scale headwater storage dams and reservoirs on stream water quality and quantity in the Canadian Prairies. *Journal of Soil and Water Conservation*, *66*(3), 158–171.

9.6 Water Quality and Sediment Data Trends

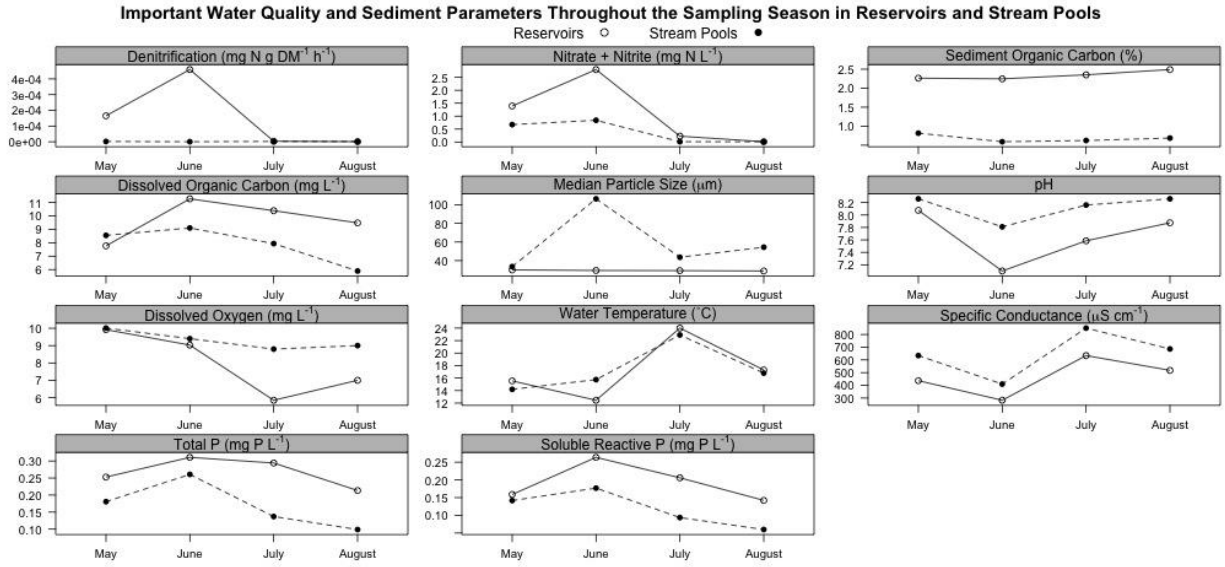

Figure A1. Median values of important water quality and sediment parameters for reservoirs and stream pools from May to August of 2013 in the Tobacco Creek Model Watershed. DM = dry matter of sediment, P = phosphorus.